ZOOLOGICA

SCIENTIFIC CONTRIBUTIONS OF THE NEW YORK ZOOLOGICAL SOCIETY

VOLUME 40 • 1955 • NUMBERS 1 TO 17

PUBLISHED BY THE SOCIETY
The ZOOLOGICAL PARK, *New York*

NEW YORK ZOOLOGICAL SOCIETY

GENERAL OFFICE
30 East Fortieth Street, New York 16, N. Y.

PUBLICATION OFFICE
The Zoological Park, New York 60, N. Y.

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Part 1. May 14, 1955

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Revision of the Genus *Cosmotoma* Blanchard. (Coleoptera, Cerambycidae, Lamiinae)

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(Text-figures 1-15)

THIS PAPER presents the results of examination of the genus *Cosmotoma* Blanchard, incurred during broad preliminary investigations of the Neotropical Acanthocinini as a whole.

The species of this genus have proved to be comparatively rare in collections, at least in series, although several new species are herein described, some of which have up to now stood under other names. The synonymy of the genus is herein rectified, having up to the present been to a large extent given wrongly in various catalogues and papers.

Grateful acknowledgment is made to the following institutions and individuals for their kindness in sending specimens for examination:—

The United States National Museum, Washington.

The American Museum of Natural History, N. Y.

The Musée Royal d'Histoire Naturelle de Belgique, Brussels.

Zoologische Staatssammlungen, Munich.

The Hincks-Dibb collection (per Dr. W. D. Hincks), Manchester.

The Dr. P. Lepesme collection, Paris.

The Dr. J. M. Bosq collection, Buenos Aires.

The specimens used for the basic investigation are in the author's collection.

I would also like to take this opportunity of expressing my very grateful thanks to the New York Zoological Society for its grant to me during 1953 from the Society's Program for Aid of Biological Research in Europe.

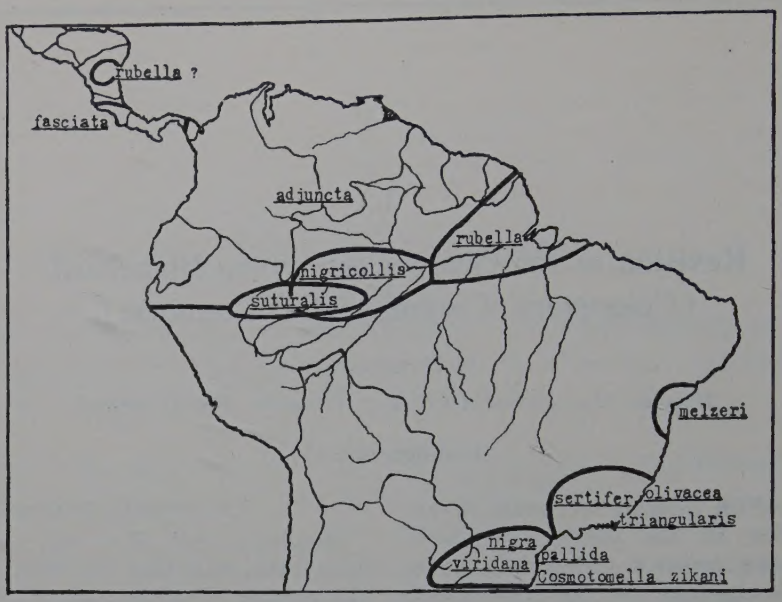
A distribution map of the genus is given (Text-figure 1) in this paper.

Cosmotoma Blanchard

Blanchard, 1845, Hist. Nat. Ins., *2*, 155.—Bates, 1864, Ann. Mag. Nat. Hist., (3) *13*, 147.—Lacordaire, 1872, Gen Col., *9* (2), 767, 780.—Bates, 1881, Biol. Centr. Amer. Col., 5, 160.

Beltista Thomson, 1860, Classif. Ceramb., 16; 1864, Syst. Ceramb., 355.

Description.—Not very robust, a little elongate-oval in shape; pubescent; with erect hairs scattered throughout. Head moderately concave between the antennal tubercles, which are not strongly raised; genae elongate; lower lobes of the eyes small, acuminate inferiorly. Antennae from about one and a third times up to about twice as long as the body in males, slightly longer than the body to about one and a half times as long in females; covered with long fine hairs, most numerous below; the fourth segment bearing apically a large distinct tuft of hairs, which often completely encircles the apex; segments two, three, and sometimes five and six with a pencil of hairs beneath the apex; the scape not very swollen, moderately elongate, only reaching to about the middle of the pronotum but equal to or longer than the third segment; the fourth segment longer than the third, (Lacordaire states that the third is longer than the fourth, but this is probably an error in transcription), the fifth to eleventh gradually decreasing in length. The pronotum transverse, convex, bearing two discal obtuse tubercles, narrowed basally and apically; the lateral tubercle on each side rather strong, conical, placed a little behind the middle. Scutellum small, sub-triangular. Elytra not very elongate, slightly less than twice as long as broad; only moderately convex, sub-parallel or moderately rounded laterally, declivous and narrowing posteriorly; projecting beyond the pronotal base a little anteriorly; obliquely truncate apically;



TEXT-FIG. 1. Distributional map of the species of *Cosmotoma* Blanchard.

with a centro-basal tumescence on each elytron, bearing a fascicule of hairs. Legs of moderate length; femora pedunculate then strongly ovularly swollen apically; tarsi short, the first segment of the posterior tarsi scarcely as long or slightly shorter than the following two united. Prosternal protuberance rather narrow, curved posteriorly; mesosternal protuberance broad, triangular, recurved posteriorly. Apical ventrite tranverse, sub-triangularly broadly rounded in male; a little more elongate in the female, and with a median longitudinal groove on the basal half.

Genotype: COSMOTOMA ADJUNCTA Thomson

The genus *Beltista* Thomson was only named by that author because he considered that the name *Cosmotoma* was too similar to the name *Cosmisoma* (Cerambycidae, Cerambycinae). It therefore is a synonym of *Cosmotoma* Blanchard.

The name *Cosmotoma* was first given by Dejean in 1836, Col. Cat., ed. *3*, 364, but as neither of the two species, *venustulum* Dejean and *plumicorne* Dupont, listed there are valid, the name does not become valid until Blanchard described it in 1845. Blanchard here gives the species *venustulum* Dejean in the genus. This name is however still invalid as no description has been made of it. In 1860, Thomson described the species *adjuncta* as genotype of his genus *Beltista*. Chevrolat then stated (1861, Journ. Ent., *1*, 188) that *adjuncta* Thomson was synonymous with *venustulum* Dejean. The latter being, however, an invalid name, and the name *adjuncta* Thomson the first available valid one, the latter is consequently the type of the genus *Cosmotoma* Blanchard. It appears that *sertifer* Serville cannot be considered under the International Code, Article 30, Section *11*, e (1).

It is interesting to note that Thomson, after his description of *adjuncta* (1860, Classif. Ceramb., 16) states: "Ma collection renferme 6 espèces devant rentrer dans ce genre." Strangely enough, however, he never described any of these and it is possible that some of the new species which I describe herein may be similar to some of those among the six mentioned.

KEY TO SPECIES

1. Elytral apex simply obliquely truncate, the marginal angle not spinously produced . 2
   Elytral apex with the marginal angle spinous 6
2. Base of elytra black 3
   Base of elytra not black, nor the suture black on the anterior half 4
3. Pronotum black from the anterior transverse groove; basal black elytral area with a ferruginous prolongation to the humerus giving the black area a bilobed appearance, the antero-sutural black portion not joining the posterior black area . . . . . *suturalis* sp. nov.

Pronotum only black on its posterior half; basal black elytral area complete, without a ferruginous prolongation to the humerus, the suture completely black between anterior and posterior black areas ..... *fasciata* Fisher

4. The pronotum completely more or less blackish in color ..... *adjuncta nigricollis* Bates
The pronotum not completely black in color, at most basally and part laterally .5
5. Pronotum completely light ferruginous in color .....*adjuncta rubella* Bates
Pronotum light ferruginous basally, extending posteriorly in a triangle to a little behind the middle, the rest blackish ..... *adjuncta* Thomson s. str.
6. Completely more or less unicolorus, without markings 7
Not unicolorus, with light and dark bands and markings 8
7. Completely blackish in color ..... *nigra* sp. nov.
Completely more or less yellowish-ferruginous in color .....*pallida* sp. nov.
8. The post-median elytral transverse black band almost straight anteriorly ..... *melzeri* sp. nov.
The post-median dark colored band not straight, distinctly curved and may be broken in part or lacking 9
9. Pronotum greenish-black or blackish in color 10
Pronotum ferruginous in color 11
10. Post-median elytral transverse band distinct, somewhat irregularly, fairly strongly, anteriorly curved, distinctly blackish pubescent and strongly contrasting from the rest of the elytra; the two discal prontal tubercles strongly separately raised; the pronotum with distinct large punctures in the anterior transverse groove ..... *viridana* Lacordaire

Post-median elytral band not present as such, not blackish, slightly posteriorly curved, but only present as the basal dermal olive-green color separated from the rest by grayish pubescence; the two discal pronotal tubercles not at all separated medially, but planely connected; no large punctures present in the anterior pronotal groove .....*olivacea* sp. nov.
11. Post-median elytral band black, narrow, distinctly strongly and regularly curved, broadly bordered posteriorly with ferruginous pubescence; the third antennal segment slightly shorter than the scape (♂ unkown) .....*triangularis* sp. nov.
Post-median elytral band almost not present, faint, ferruginous pubescent in part at the suture; the third antennal segment slightly longer than the scape (♀♀ unknown) ..... *sertifer* Serville

Cosmotoma adjuncta Thomson

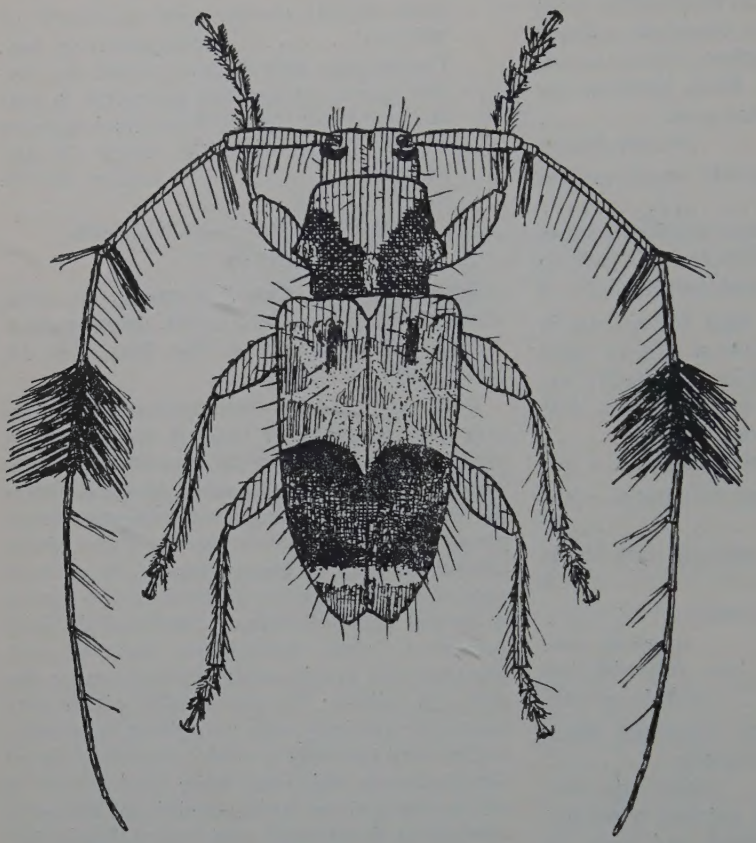
Text-fig. 2

Thomson, 1860, Classif. Ceramb., 16.—Chevrolat, 1861, Journ. Ent., *1*, 188. (nota synon.).—Bates, 1864, Ann. Mag. Nat. Hist., (3) *13*, 148. (nota).

Description. Male.—Ferruginous in color except for the posterior half of the pronotum, pitchy, extending forward laterally from the middle and narrowing to about the basal fifth at the border, thus leaving a sub-triangular ferruginous area medio-anteriorly; the elytra pitchy behind the middle, except usually the extreme apex, the anterior border of this dark area strongly curved and well defined; the basal declivity somewhat lighter ferruginous than the rest. Marked with grayish-white pubescence on the elytra as follows: the post-median pitchy area bordered anteriorly with grayish-white, ramifying forward suturally in a fairly regularly placed lattice-work in which one white band extends to the margin at about the basal third, one obliquely forward to the humeri, and one obliquely forward to the suture at about the basal quarter; a distinct transverse white fascia at about the apical sixth; the centro-basal elytral tumescence crested with strong black setae. The antennal segments from the third with grayish pubescence basally, the setae, fasciculae and brushes of hairs black.

The underside darker ferruginous than above, sometimes pitchy-brown and the abdomen occasionally almost black. Covered with grayish-white pubescence, thin in parts and most dense on the sides of sternum and laterally on the posterior border of the first abdominal segment.

Antennae one and a half to one and three-quarter times as long as the body; slender; fringed below, after the fourth segment chiefly at the apices; segments two and three with distinct pencils of black hairs beneath, the latter at the apex; the fourth segment bearing on a little more than its apical third a strong dense brush of long hairs, which encircles the segment, which is somewhat swollen on the area which bears the setae; the scape moderately elongate, reaching to about the middle of the pronotum, a little swollen, but not strongly; the third segment about one and a third times as long as the scape, the fourth segment almost one and a



TEXT-FIG. 2. *Cosmotoma adjuncta* Thomson s. str. ♂ (× 9.6).

half times as long as the third segment, about twice as long as the scape, and about four times as long as the fifth segment, the rest gradually decreasing to the apex; the segments completely finely and closely punctured, except where the fasciculae and brushes of setae arise where the punctures are very much larger. The antennal tubercles only slightly raised, the head broadly and slightly concavè between; the frons large, about equilateral, moderately convex, with a very fine median longitudinal groove; the lower lobes of the eyes small, narrowing inferiorly, about one and a half times as long as broad, about equal in length to the genae; the head completely finely and closely punctured, bearing a few long setae anteriorly and at the inner frontal margin of the eyes; finely tawny pubescent.

The pronotum transverse, convex, with two, well raised, obtuse tumescences on the disc; with a strong broadly conical, spinous swelling laterally on each side slightly post-medially; completely very finely and closely punctured, with a single row of sparse, very large punctures apically and one basally in the transverse grooves, which are broad and rather shallow; finely tawny pubescent anteriorly, grayish pubescent posteriorly on the dark area, most dense on the posterior border of the lateral spines. The scutellum sub-triangular to sub-rotundate, fairly narrowly rounded apically; very finely and closely punctured; finely grayish pubescent.

The elytra not very elongate, only moderately convex, rounded laterally; the apices obliquely truncate, the marginal angle not spinous; the centro-basal crests strongly raised, black fasciculate; completely finely and closely punctured, the scattered black setae rising from larger punctures.

The prosternal protuberance narrow, particularly between the coxae, somewhat broadly concave medially; strongly curved. The mesosternal protuberance very broad anteriorly, sub-triangular, truncate apically, the truncature being somewhat wider than the breadth of the prosternal protuberance medially. The ventrites of normal size; the apical segment transverse, somewhat sub-triangular and broadly rounded apically. The underside completely very finely and variably closely punctured; finely grayish-white pubescent, mostly rather sparse, most dense on the sides of the metasternum and latero-posterior border of the first abdominal segment.

The legs of moderate length; femora strongly pedunculate; tarsi moderately slender, the first segment of the posterior about equal in length to the following two segments united; all the legs very finely and fairly closely punctured; sparsely and finely grayish-tawny pubescent.

Female.—Similar in color to the male. The antennae a little less elongate. The apical ventrite a little more elongate.

Length, 6.5-8 mm.; breadth, 2.5-3 mm.

Locality.—French Guiana—(Chevrolat) (Mus. Hist. Nat. Belg.). Brazil—Para (Gilmour coll.) (1 ♂); "Amazon" (Mus. Hist. Nat. Belg.); Santarem (United States National Museum) (1 ♂, 4 ♀); Manaos (Hincks-Dibb coll.) (1 ♂). Peru—Achinamiza (1.XII.26, H. Bassler) (Amer. Mus. Nat. Hist.) (1 ♀); Callanga (Hincks-Dibb coll.) (1 ♂). Colombia—Cartagena (1.1.21) (Amer. Mus. Nat. Hist.) (1 ♀).

Material Examined.—Mus. Hist. Nat. Belg., 2; Amer. Mus. Nat. Hist., 2; Gilmour collection, 1; United States National Mus. 5; Hincks-Dibb collection, 2; Total: 12.

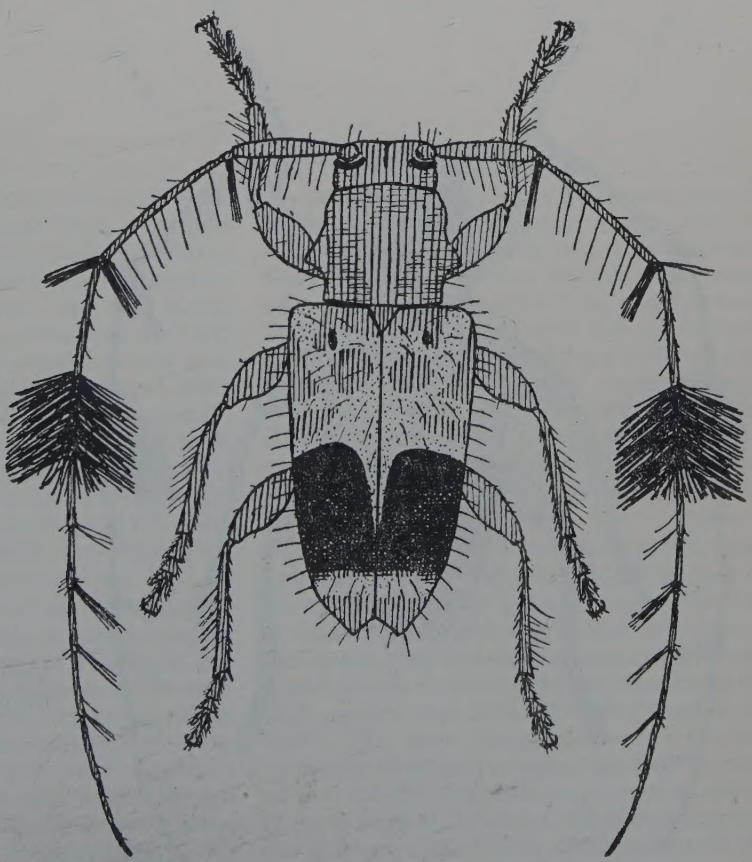
COSMOTOMA ADJUNCTA Thomson var. (?subsp.) RUBELLA Bates

Text-fig. 3

Bates, 1864, Ann. Mag. Nat. Hist., (3) *13*, 14; 1872, Trans. Ent. Soc. Lond., 237; 1881, Biol. Centr. Amer. Col., 5, 160, pl. 13, fig. 8.

Cosmotoma rubella Bates has stood up to the present as a good species but so far as I can ascertain it is structurally quite similar to *Cosmotoma adjuncta* Thomson and the distribution areas of *adjuncta* Thomson and *rubella* Bates overlap. Bates, however, states (1872, Trans. Ent. Soc. Lond., 237) that the specimen from Chontales, Nicaragua, is "rather darker in colour of the elytra than specimens from the Amazons" and in my opinion, in view of the fact that there is such a wide gap between the known distributional areas, this specimen might be of a different species. The coloration of the figure given by Bates is not particularly good.

Description.—The variety *rubella* Bates differs most conspicuously from the typical form of *C. adjuncta* Thomson in the pronotum being completely ferruginous-red in color, without any trace of dark color anywhere. This would appear to be quite constant. Also the posterior pitchy area of the elytra seems to have its anterior curved border continued suturally a little further behind before meeting the suture.



TEXT-FIG. 3. *Cosmotoma adjuncta rubella* Bates. ♂ (× 11.0).

Length, 5-7.5 mm.; breadth, ?-2.8 mm.

Locality.—Brazil—Para (Bates); R. Tapajos (Bates). French Guiana—(Bates); (Gilmour collection) (1♀). Nicaragua—Chontales (Belt) (Bates).

Material Examined—Gilmour collection, 1.

COSMOTOMA ADJUNCTA Thomson, var. (?subsp.) NIGRICOLLIS Bates

Text-fig. 4

Bates, 1864, Ann. Mag. Nat. Hist., (3) *13*, 148.

Cosmotoma nigricollis Bates, which has previously been sunk as a synonym of *Cosmotoma adjuncta* Thomson, has in my opinion as good a claim to validity as *C. rubella* Bates. In the same way that *rubella* Bates is a very distinct form of *Cosmotoma adjuncta* Thomson, so I consider *nigricollis* Bates to be equally distinct, but being the darker colored form and not lighter form as *rubella* Bates. Structurally again I can find no distinguishing feature for *nigricollis* Bates from *C. adjuncta* Thomson or *rubella* Bates.

Description.—The variety *nigricollis* Bates differs most conspicuously from the typical form of *C. adjuncta* Thomson in the pronotum being completely pitchy-black in color without any ferruginous color at all. The posterior half of the head is also dark colored. This coloration is constant in all specimens examined. Also the preapical transverse white pubescent band on the elytra is often more extensive, though the pubescence is thinner, thus giving the appearance of a rather narrower posterior elytral dark colored area. This form is shown in the figure given herein.

Length, 5.5-6.5 mm. (Bates' specimens appear to have been larger, measuring 7.7-8.5 mm.); breadth: 2.2-2.5 mm.

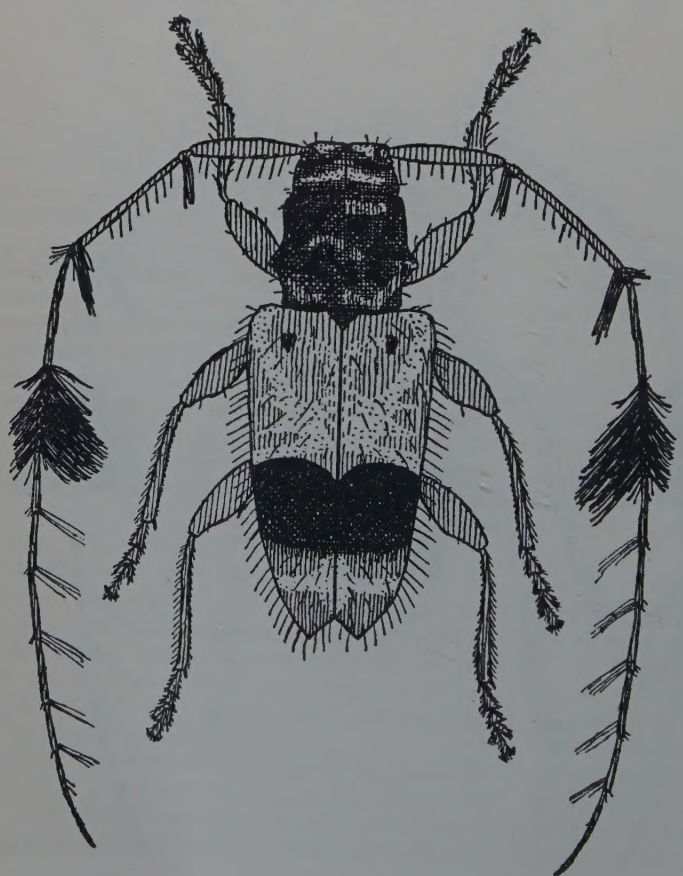
Locality.—Brazil—Upper Amazons, Ega (Bates); Amazon (Gilmour coll.) (1♀), (R. Mus. Nat. Hist. Belg.); (Am.m.!) (Zool. Staatssamml., Mun.) (1♂); Teffe (X1.24, H. Bassler) (Amer. Mus. Nat. Hist.) (1♂).

Material Examined.—Amer. Mus. Nat Hist., 1; Mus. Hist. Nat. Belg., 1; Zool. Staatssamml., Mun., 1; Gilmour collection, 1; Total: 4.

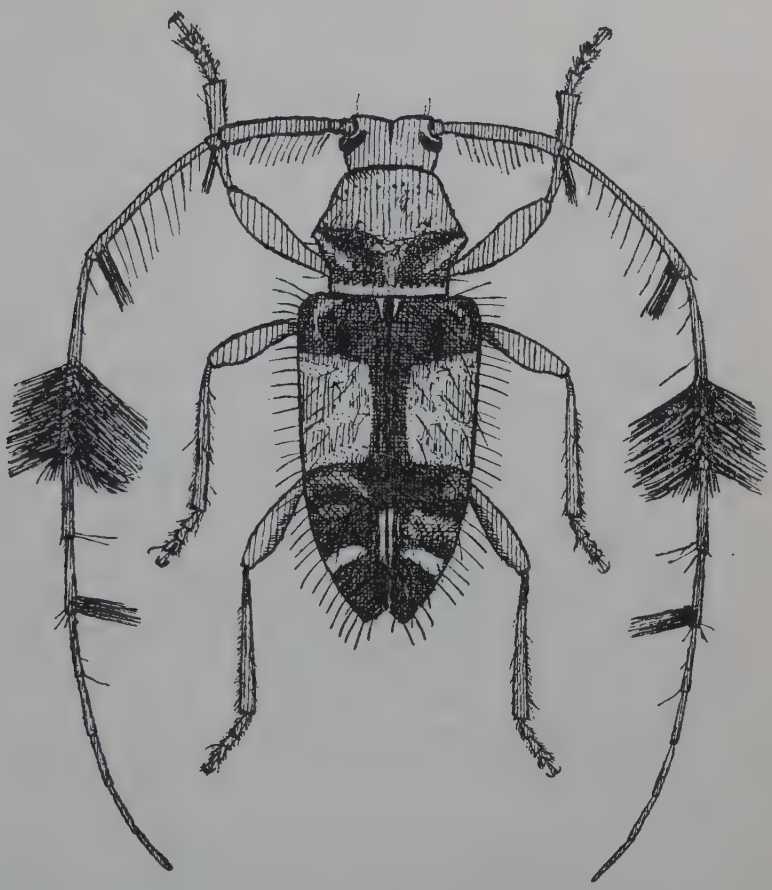
COSMOTOMA FASCIATA Fisher

Text-fig. 5

Fisher, 1931, Journ. Wash. Acad. Sci., *21* (2), 23.



TEXT-FIG. 4. *Cosmotoma adjuncta nigricollis* Bates. ♂ (× 11.0).



TEXT-FIG. 5. *Cosmotoma fasciata* Fisher. ♂ (× 9.6). Paratype.

Description. Male.—Ferruginous and black in parts as follows: antennae ferruginous, the setae black; the head ferruginous. Pronotum with about the anterior half and posterior border (narrowly) ferruginous, the rest black; scutellum black. The elytra with almost the basal quarter black, the posterior border of this area almost rectilinear, and about the apical two-fifths black (the anterior border only slightly curved), these two areas united by a common sutural black band; the area between these ferruginous. The legs ferruginous, a little darker basally; the anterior femora pitchy postero-dorsally. The ventral surface pitchy to black, except the head and anterior border of the pronotum.

The head and anterior half of the pronotum with sparse tawny pubescence, this becoming denser towards the sides of the pronotum and forming a vague macula dorso-laterally on each side. Marked with thin white or grayish-white pubescence as follows: the pronotum medially longitudinally and behind the lateral tubercles on the posterior half; the lateral borders of the scutellum; the inner side of the humeri; three somewhat curved irregular transverse fasciae on the ferruginous elytral area which unite towards the suture; one or two vague transverse areas on the apical black area, and a very distinct densely white pubescent oblique latero-discal macula at about the apical fifth, and an almost as distinct, though less clearly defined, common white sutural macula at about the apical quarter. The underside more or less completely very sparsely grayish-white pubescent, which becomes densely white at the latero-posterior angles and posterior border of the sternum and at the latero-posterior borders of the first and apical abdominal segments.

Antennae about one and three-quarter times as long as the body; slender; fringed beneath on the basal segments; the fourth segment bearing round its apical half a large brush of black hairs; the second, third and sixth segments with distinct apical pencils of black hairs, (Fisher states the fifth segment, not sixth, but this I think is an error). The scape elongate, not very swollen, extending to about the apical third of the pronotum; the third segment about one and a third times as long as the scape, the fourth segment not quite one and a sixth times as long as the

third, about one and two-third times as long as the scape, and about two and a third times as long as the fifth segment, the rest gradually decreasing to the apex; the fourth segment distinctly swollen on its apical half; the antennal segments completely and finely punctured, with larger punctures from which the setae arise, particularly on the fourth segment.

The antennal tubercles slightly, but not strongly, raised, slightly broadly concave between; the frons about as long as broad, slightly convex, with a fine median longitudinal groove; the lower lobes of the eyes small, narrowing inferiorly, slightly longer than broad, about equal in length to the genae; the head completely very finely and closely punctured, with a few long erect setae scattered marginally on the frons, particularly at the anterior border.

The pronotum slightly broader than long, convex, with two well raised obtuse tumescences on the disc; with a slight, though broadly conical, spinous swelling laterally on each side, slightly behind the middle; completely very finely and closely punctured, with a few large coarse punctures in the anterior and posterior transverse grooves, which latter are broad and only moderately deep. The scutellum a little longer than broad, sub-triangular, very broadly rounded, almost truncate, apically; very finely and closely punctured.

The elytra moderately elongate, moderately convex; almost parallel-sided laterally to about the apical third, then broadly rounded to the apices, which are obliquely internally truncate, the marginal angle not spinous; the centro-basal tubercle on each elytron strongly raised, black fasciculate above; completely finely and closely punctured, with a number of larger, coarse, punctures scattered here and there, from which erect black setae arise; a distinct sub-sutural line on each elytron, which is much less distinct premedially.

The prosternal protuberance moderately narrow, more so medially, longitudinally concave medially, strongly curved. The mesosternal protuberance subtriangular, very slightly tumescent medially, strongly curved, truncate apically, the truncature being only very slightly broader than the breadth of the prosternal protuberance medially. The apical ventrite very broadly somewhat subtriangular in shape, the apex broadly rounded. The whole underside completely finely and closely punctured; the apex of the apical ventrite with a few large coarse punctures.

The legs of moderate length; the femora strongly pedunculate; the tarsi fairly slender, the first segment of the posterior about equal in length to the following two united; all very finely and fairly closely punctured.

Female.—Apparently unknown. (I have only seen one male paratype and from Fisher's original description it would appear that all the specimens are males).

Length, 5-7.5 mm.; breath: 2-3.2 mm.

Locality.—Costa Rica—Reventazon River, Hamburg Farm, (4.11.25, F. Nevermann) (Type Locality). Panama—(15.IV.37), In Banana debris (W. J. Fisher *in litt.*).

Type, two Paratypes and one other specimen (Panama) in the United States National Museum. (The F. Nevermann collection was purchased by this institution on Nevermann's death). One Paratype (♂) in the Gilmour collection. (Exchanged with the United States National Museum for a paratype of *Cosmotoma pallida* sp. nov.).

This species is most closely allied to *Cosmotoma suturalis* sp. nov. described in this paper, the differences from it being given with that species. From *Cosmotoma adjuncta* Thomson and the other species it differs at first glance in both base and apex of the elytra being black.

Cosmotoma suturalis sp. nov.

Text-fig. 6

Description.—Male. Ferruginous and black in parts as follows: antennae ferruginous, the setae black; the head ferruginous, a little darker posteriorly; the genae black. Pronotum with the anterior border ferruginous, in front of the anterior transverse groove, and the basal margin ferruginous, not extending to the posterior transverse groove medially, the rest black; scutellum pitchy-ferruginous, not quite black. The elytra black basally, almost up to the basal quarter, and extending in a first broadening, then narrowing band along the suture to about the apical two-fifths; the elytra then ferruginous up to about the apical two-fifths, with a ferruginous curved projection anteriorly to the base running on the inner side of the humeri; about the apical two-fifths black, the anterior border distinctly curved. The legs ferruginous, blackish basally and ventrally on the anterior femora, and ventro-basally on the intermediate and posterior femora.

Marked with white or grayish-white pubescence as follows: the lateral margins of the frons and the posterior border of the head; the anterior border of the pronotum thinly yellowish-white; the postero-superior border of the lateral tubercles; a triangular area on the pronotal disc, between the tumescences, apex towards the base; the elytra with whitish pubescent bands, on the ferruginous area, running from anterior border, middle and posterior border marginally to unite on the disc before the sutural black area and continued forward to the base along the inner side of the humeri; a distinct trans-

TEXT-FIG. 6. *Cosmotoma suturalis* sp. nov. ♂ (× 10.0). Holotype.

verse white fascia at about the apical fifth; a vague oblique patch of tawny pubescence on the black area in front of this.

Underside completely black, the apical ventrite slightly lighter in color—pitchy-ferruginous apically; covered thinly with grayish pubescence, which becomes dense at the latero-posterior angle of the sternum and at the side of the first and apical abdominal segments.

Antennae slightly more than one and a half times as long as the body; slender, fringed beneath on the basal segments, most densely on the second and apex of the third segments, becoming sparse apically from the fifth segment; the fourth segment bearing around its apical half a large brush of black hairs. The scape moderately elongate, a little swollen, reaching to about the basal third of the pronotum; the third segment about one and a quarter times as long as the scape, the fourth segment about one and a third times as long as the third, about one and three-quarter times as long as the scape, and nearly three times as long as the fifth segment, the rest gradually decreasing to the apex; the fourth segment distinctly swollen towards the apex where the brush of setae rises; the fifth segment rather distinctly curved; the antennal segments completely and finely punctured, with larger punctures from which the setae arise, particularly those on the fourth segment.

The antennal tubercles slightly raised, but not strongly, moderately strongly broadly concave between; the frons very slightly longer than broad, almost equilateral, slightly convex, with a fine median longitudinal groove; the lower lobes of the eyes small, a little narrowing inferiorly, slightly longer than broad, about three-quarters as long as the genae; the head completely very finely and closely punctured, with a few long setae scattered marginally on the frons; sparsely grayish-white pubescent in the main.

The pronotum transverse, convex, with two well raised, obtuse tumescences on the disc; with a strong, broadly conical, spinous swelling laterally on each side slightly post-medially; completely finely and closely punctured, with posterior transverse grooves, which latter are broad and not very deep. The scutellum slightly longer than broad, sub-triangular, broadly rounded apically; very finely and closely punctured; blackish pubescent, with the lateral borders finely grayish pubescent.

The elytra not very elongate, only moderately convex; almost straight-sided laterally to about the apical third, then broadly rounded to the

apices, which are obliquely truncate, the marginal angle not spinous; the centro-basal tumescences strongly raised, very sparsely black setose; (I believe that in the specimen examined the fasciculae have been knocked off to some extent. The setae, composing the normal fasciculae in the species of this genus, appear to be loosely attached and are easily dislodged); completely finely and closely punctured, the scattered black setae arising from slightly larger punctures; a fairly distinct longitudinal sutural carina on the apical half.

The prosternal protuberance narrow, particularly medially, strongly concave, strongly curved. The mesosternal protuberance very broad, particularly anteriorly, sub-triangular, slightly tumescent medially, truncate apically, the truncature being almost twice as broad as the breadth of the prosternal protuberance medially. The apical ventrite transverse, broadly slightly sub-triangular in shape, the apex broadly rounded. The whole underside completely finely and closely punctured; the apex of the apical ventrite with a few moderately large punctures.

The legs of moderate length; the femora strongly pedunculate; the tarsi moderately slender, the first segment of the posterior about equal in length to the following two united; very finely and closely punctured.

Female. Similarly colored to the male. More robust. The antennae about one and a half times as long as the body.

The apical ventrite transverse, but slightly sub-conically triangular in shape, with a fine median anterior groove, the apex broadly bisinuately truncate; the apex with a number of very large, coarse, close punctures.

Length, 6.5-8 mm.; breadth, 2.5-3.2 mm.

Locality.—Brazil—Manaos (♂) (Holotype); "Amazonas" (♀) (Allotype). Peru—Gancartambo (♂) (Paratype).

Holotype, ♂, in the Hincks-Dibb collection. Allotype, ♀, in the Musée Royale d'Histoire Naturelle de Belgique. (Coll. Achard) (Coll. Le Moult Box M.669) (R. Mus. Hist. Nat. Belg. I.G. 12, 595). Paratype, ♂, in the Dr. P. Lepesme collection, Paris.

Diagnosis.—This beautiful new species is most closely allied to *Cosmotoma fasciata* Fisher from Costa Rica, from which it differs in the sutural black band on the anterior half not reaching the apical black area; with a prolongation of ferruginous color to the humerus; the pronotum being almost completely black, except anterior to the transverse groove, and other differences. From *C. adjuncta* Thomson and all the other known species in the genus it differs conspicuously in markings, through the sutural black band, pronotal dark and ferruginous areas, etc.

The paratype is in much worse condition and of less bright coloration than the holotype and allotype, probably due to age and dust.

Cosmotoma sertifer Serville

Text-fig. 7

Serville, 1835, Ann. Soc. Ent. France, *4*, 59.—Lacordaire, 1872, Gen. Col., *9* (2), 654, nota 2.—Aurivillius, 1923, Col. Cat. Ed. Junk-Schenkling, *74*, 419.—Melzer, 1927, Rev. Mus. Paulista, *15*, 575 (nota synon.).—Linsley, 1933, Pan-Pacific Ent., *9* (3), 132. (Synonymy).

brasiliensis Plavilstshikov, 1927, Encycl. Ent. B.1.—Col., *2* (2), 59.

setifer Aurivillius, 1923, Col. Cat. Ed. Junk-Schenkling, *74*, 334. (*Pogonocherus*).

I do not agree with any of the other authors, viz., Aurivillius (1923), Melzer (1927), Linsley (1933), or Blackwelder (1946, Bull. U. S. Nat. Mus., *185*, 617), that *Cosmotoma viridana* Lacord. is synonymous with *Cosmotoma sertifer* Serville. I regard them as two distinct species.

C. sertifer Serville has not been described or even mentioned as a collected species from the time it was described and although Serville's original description lacks many important points in view of modern knowledge, there are several points of note which could not have been missed out of any description, however early, particularly as regards coloration. Most important and immediately noticeable is the lack of mention of any post-median black transverse band. I have seen the types of *viridana* Lacord., and this band is distinctly present. I have further seen a single specimen which agrees with the description of *sertifer* Serville and it is quite distinct from *viridana* Lacordaire.

I give a translation of Serville's original description below for comparison, as well as a full description of the specimen examined.

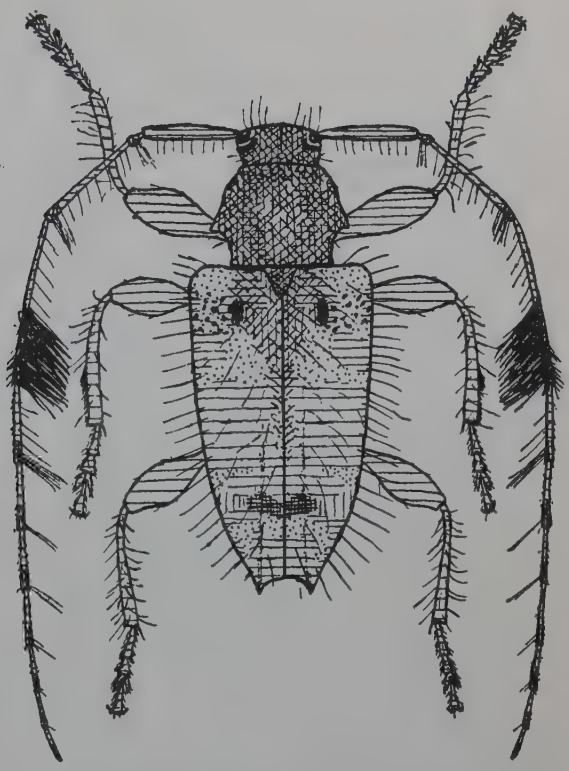
Serville placed his species *sertifer* in his 1st Division of the genus *Pogonocherus*, separated by the following characters:

"Elytra truncate at the apex; the external angle of the truncature unispinous (p. 57).

"(Length, 2 to 3 lines [i.e., ca. 4.2-6 mm.]).

"Body a little shining, blackish and covered with long brown hairs, sparse, above; ferruginous below. Pronotum swollen at the middle, bordered posteriorly. Elytra slightly bordered exteriorly and at the suture, each having at the base a feeble tubercle bearing some long stiff and brown hairs; they are tinted with greenish towards their apex. The antennae brown, having a tuft of black hairs on their fifth segment, the first greenish. The legs of ferruginous brown, with some hairs like the body; femora greenish.

"From Brazil. My collection." (Translation of original description).



TEXT-FIG. 7. *Cosmotoma sertifer* Serville. ♂ (× 10.3).

There is a very obvious discrepancy in this description where it states that the *fifth* segments of the antennae bear a tuft of black hairs. This I believe is almost certainly a mistake. If it is not an error on the part of author or printer, then the species of Serville does not belong to this genus, and the specimen which I describe below will need a new name in being a new species.

Neither does Serville state the extent of the brownish pubescence or greenish color of the elytra. I take it that the head and pronotum are brown pubescent and the elytra in part, gradually becoming green towards the apex. In my specimen the elytra are almost wholly greenish, but I presume that this is perhaps a rather variable character.

Male.—Ferruginous and greenish as follows: head and pronotum closely ferruginous pubescent, the extreme apex of the scape and its second segment completely ferruginous, the tarsi in the main ferruginous, the underside completely ferruginous, the sternum however pitchy-red; the antennae in the main, and the femora and tibiae greenish, the elytra greenish in the main except baso-suturally and narrowly along the suture, and a vague, ferruginous, irregular, transverse band at about the apical third, the elytra also with vague areas of silvery-gray pubescence, chiefly on the inner side of the humeri extending posteriorly to about the basal third, thence branching to margin and suture, running narrowly along the latter and extending to the margin again just before the apical third, and apically. The underside with thin grayish pubescent, a little more dense on the sternum and sides of the anterior abdominal segments.

Antennae about one and two-thirds times as long as the body; slender, fringed beneath, becoming sparse after the fourth segment, where they are chiefly confined to the apices; segments two, three and five with thin pencils of hairs beneath the apex, segment four bearing a strong brush of dense black setae on the inner apical two-fifths, not extending completely round the segment; which is somewhat swollen on the area from which the setae arise. The scape moderately elongate, reaching to about the apical quarter of the pronotum, a little swollen; the third segment about one and a fifth times as long as the scape, the fourth segment about one and a half times as long as the third, about one and three-quarter times as long as the scape, and twice as long as the fifth segment, the rest gradually decreasing to the apex; the segments completely finely and fairly closely punctured, with somewhat larger punctures from which the long setae arise. The antennal tubercles only slightly raised, the head broadly and only very slightly concave between; the frons large, more

or less equilateral, moderately convex, with a very fine median longitudinal groove; the lower lobes of the eyes very small, almost subquadrate, only slightly narrowing inferiorly, about three-quarters as long as the genae; the head completely finely and closely punctured, bearing a number of distinct tawny long setae at the inner border of the lower lobes of the eyes and lower border of the frons; completely finely and fairly closely ferruginous pubescent.

The pronotum transverse, moderately convex, with two strongly raised conical discal tumescences; with a moderately strong, broadly conical spinous tubercle on each side slightly behind the middle; competely finely and closely punctured with a number of fairly close, extremely large, scattered punctures in the anterior and posterior transverse grooves, which are broad and very shallow, except a little deeper medially; completely uniformly ferruginous pubescent, with a slight silky sheen in certain lights. The scutellum sub-triangular, extremely broadly rounded apically, almost truncate; very finely and closely punctured; dark ferruginous pubescent, lighter marginally.

The elytra not very elongate, only moderately convex, slightly rounded laterally, the apices obliquely truncate, the marginal angle distinctly spinously produced; the centro-basal crests strongly raised, black fasciculate; the elytra completely finely and closed punctured, with an irregular longitudinal band of extremely large punctures running from the inner side of the humerus along the outer side of the centro-basal tubercle almost to the middle of each elytron, a few slightly smaller and more sparse punctures on the sutural side of the centro-basal tubercle and a number of still slightly smaller punctures, more scattered, on the lateral and apical half of the elytra, from some of which the long, erect setae arise.

The prosternal protuberance narrow, particularly between the coxae, where it is sub-parallel in the main, strongly longitudinally concave, strongly curved. The mesosternal protuberance extremely broad anteriorly, sub-triangular, truncate and very slightly emarginate apically, the truncature only a little wider than the breadth of the prosternal protuberance medially. The apical ventrite strongly transverse, a little sub-triangular, the apex extremely broadly rounded and very slightly emarginate apically. The underside completely very finely and, in the main, closely punctured; finely grayish pubescent, sparse in the main, but a little more dense on the sternum and sides of the anterior abdominal segments.

The legs of moderate length; femora very strongly pedunculate; tarsi moderately slender, the first segment of the posterior tarsi about equal in length to the following two united; all the legs very finely and moderately closely punctured; sparsely grayish pubescent.

Female.—Apparently unknown. (As *C. viridana* has up to the present been synonymous with this species, it is possible that specimens of the two species are mixed together in collections. Serville does not state the sex of his specimens).

Length, 6 mm.; breadth, 2.2 mm.

Locality.—Brazil—(Serville); Rio de Janiero (Gilmour coll.) (1 ♂).

Material Examined.—Gilmour collection, 1.

This is one of the smaller species of the genus.

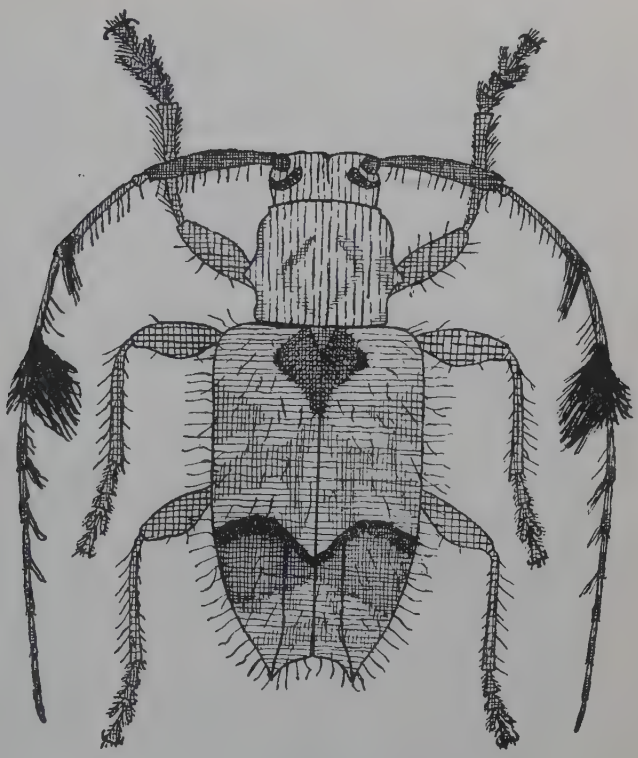
The specimens listed by Bosq (1944, Rev. Soc. Ent. Argent., *12*, 201) from the Argentine are *C. viridana* Lacordaire, and not *C. sertifer* Serville. Dr. Bosq has sent me for examination a typical specimen which is certainly *viridana* Lacordaire.

Cosmotoma triangularis sp. nov.

Text-fig. 8

Description.—Female. Dark-brown to ferruginous and olive-green as follows: head and pronotum closely ferruginous pubescent; the elytra in the main olivaceous in color, except for some variegated silky gray pubescence around the humeri, extending to behind the centro-basal tubercles, then brokenly running to the margin at about the middle, a little suturally, and a little bordering the post-median dark band anteriorly and posteriorly, with the scutellum dark brown and the area from the base of the scutellum to the apex of the centro-basal tubercles thence extending to the suture at about the basal quarter, dark brown, with a little ferruginous pubescence, thus giving a triangular mark on each elytron, its base the suture, its apex at the centro-basal tubercle; also on each elytron, at about the apical two-fifths, a very distinct transverse, narrow, curved, dark-brown band, this shading off posteriorly to lighter brown, with tawny pubescence, to about the apical fifth to sixth; the antennae greenish in color, with the extreme apices of each segment dark ferruginous; the legs dark olivaceous, the tarsi dark brown; the underside pitchy-black, with the apex of the last ventrite ferruginous, covered with thin grayish pubescence, which is most dense on the sternum and the sides and posterior borders of first and second abdominal segments.

Comparatively (in this genus) robust, rather broadly elongate-ovate. Antennae only a little longer than the body (about one-ninth), (unfortunately lacking after the third segment in the Paratype), moderately slender, fringed beneath up to the sixth segment, with thin pencils of



TEXT-FIG. 8. *Cosmotoma triangularis* sp. nov. ♀ (× 7.0). Holotype.

setae at the apices of the third and fifth to seventh segments, the fourth segment bearing a large distinct brush of black setae on almost its apical half, which does not completely encircle the segment, but only about the longitudinal minor half, the area from which the setae of the brush arise being somewhat swollen. The scape moderately elongate, extending to about the basal fifth of the pronotum, not very swollen; the third segment about a fifth *shorter* than the scape, the fourth segment almost one and a half times as long as the third, only about one and a sixth times as long as the scape, and twice as long as the fifth segment, the rest gradually decreasing to the apex; the segments completely finely and fairly closely punctured, with distinctly larger punctures from which the setae arise. The antennal tubercles only slightly raised, the head very broadly and slightly concave between; the frons large, about equilateral, only moderately convex, with a very fine median longitudinal groove; the lower lobes of the eyes very small, slightly narrowing inferiorly, about as long as broad, about two-thirds as long as the genae; the head completely finely and closely punctured, bearing a few long setae at its anterior border and at the inner margin of the lower lobes of the eyes.

The pronotum transverse, moderately convex, bearing two strongly raised conical obtuse tumescences on the disc; with an only moderately strong, conical, narrowly obtuse, tubercle laterally on each side slightly behind the middle; completely finely and closely punctured, with a number of very large punctures in a somewhat irregular more or less single row in both anterior and posterior transverse grooves, and extending more sparsely between the discal tubercles, and a few on the inner side of the base of the lateral tubercles; the anterior and posterior transverse grooves broad, and very shallow. The scutellum sub-triangular, very broadly rounded apically; very finely and closely punctured.

The elytra not very elongate, only moderately convex, more or less parallel-sided laterally to behind the middle thence broadly rounded to the apices, which are obliquely truncate, with the marginal angle rather stoutly and strongly spinously produced; the centro-basal tumescences moderately strongly raised and black fasciculate; the elytra finely and closely punctured, with a number of variably close, scattered, slightly larger punctures here and there and some much larger sparse punctures from which the long, erect setae arise.

The prosternal protuberance narrow, particularly between the coxae, where it is sub-parallel, rather strongly longitudinally concave, strongly curved. The mesosternal protuberance extremely broad and rather swollen anteriorly, sub-triangular, the apex truncate and very slightly emarginate apically, the truncature about equal in

width to the breadth of the prosternal protuberance medially. The ventrites of normal size; the apical segment transverse, broadly rounded laterally, the apex broadly emarginate, the lateral angles rounded, bearing a median longitudinal groove on the anterior half. (In the Holotype this is rather obtuse and ill-defined; in the Paratype it is much more distinctly marked). The underside completely finely, and in general, closely punctured, with a number of large, rather close punctures at the apex of the apical ventrite from which distinct setae arise.

The legs of moderate length; the femora strongly pedunculate; the tarsi moderately slender, the first segment of the posterior tarsi about equal in length to the following two united; all the legs very finely and fairly closely punctured; sparsely grayish pubescent.

Length, 10-10.25 mm.; breadth, 3.75-4 mm.

Locality.—Brazil—Rio de Janiero (Holotype); (Paratype).

Holotype, ♀, and Paratype, ♀, in my collection.

Diagnosis—This new species is easily the largest and most robust so far known in the genus. It appears to be most closely allied to *Cosmotoma sertifer* Serville (of which I feel quite certain it is not the female), but differs conspicuously by its large size, possessing a distinct postmedian dark colored transverse elytral band, in the underside being black with the extreme apex ferruginous and the third antennal segment shorter than the scape, etc.

Cosmotoma viridana Lacordaire

Text-fig. 9

Lacordaire, 1872, Gen. Col., 9 (2), 781, nota 1, p. 108, fig. 4 (non 3).

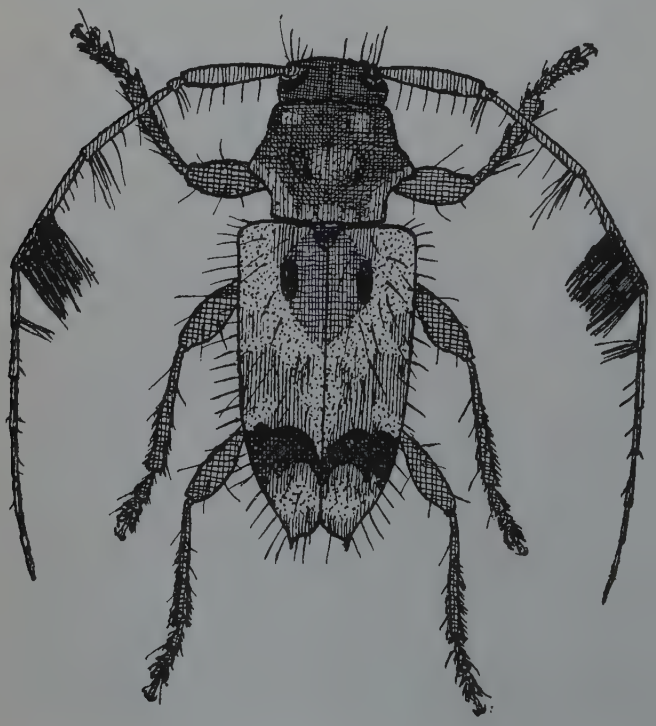
sertifer Bosq, 1944, Rev. Soc. Ent. Argent., *12*, 201.

This species is certainly, in my opinion, quite distinct from *Cosmotoma sertifer* Serville with which it has been consistently synonymised since its description, with the exception of Gemmiger & Harold (1873, Cat. Col., *10*, 3153) who, however, did not have *C. sertifer* Serville in the genus *Cosmotoma,* but retained it in *Pogonochaerus* (sic!) (l.c 3117). Apart from that it has been always synonymised by other authors who considered the two to be identical. I further believe that Argentinian specimens listed as *sertifer* Serville are almost certainly *viridana* Lacordaire.

I am very grateful to the authorities of the Musée Royale d'Histoire Naturelle de Belgique for so kindly sending me the Type specimen of Lacordaire, which is in excellent condition.

In view of the fact that this species and *Cosmotoma sertifer* Serville have been for so many years confounded, and that I have given a translation of Serville's original description, I give also, below, a translation of Lacordaire's original description.

"Gray-green, dark beneath, above silky and dark mixed, tarsi yellowish; 3rd antennal seg-



Text-fig. 9. *Cosmotoma viridana* Lacordaire. ♀ (× 8.0). Type.

ment not penicillate; disc of prothorax strongly binodose; elytral apices spinose, with a single obtuse costa running down from the middle to the apex and provided with a black fasciculate basal crest, a common fascia behind the middle dark colored. Length, 8 mm. Habit. Brazil (Santa-Catherina Island). A very close species, perhaps the same, exist in some collections under the name of *pulchellum* Chevrol." (Translation of original description).

The Type of Lacordaire's description is a female and the figure given is of this specimen.

Description. Male.—Dark pitchy-brown and greenish intermixed; the head and pronotum pitchy; the elytra dark brown baso-suturally, covering the centro-basal crests and with a very distinct somewhat undulating transverse blackish-brown fascia at about the apical third; the pronotum with grayish pubescence postero-laterally from the apex of the lateral spines to the base; the elytra with variegated silky grayish pubescence with the green dermal color intermixed, chiefly grayish from the humeri almost to the middle, except on the baso-sutural dark area, and then apically behind the transverse fascia, medially between more broken and much less regular and extensive. The underside pitchy-black to dark ferruginous; covered with thin grayish pubescence which is most dense on the sternum and sides of the first abdominal segment.

Antennae about one and a third times as long as the body; slender; fringed below, most densely at the apices, and chiefly at the apices on the fifth to ninth segments; segment two with a pencil of setae beneath, segments three and five to seven with apical pencils of setae beneath, becoming much more sparse towards the latter; the fourth segment bearing, on about the apical two-fifths, a strong dense brush of long setae which is on the inner longitudinal half of the segment, not completely encircling the apical portion, which is swollen on the area from which the setae arise; the scape moderately elongate, extending to about the middle of the pronotum, a little swollen; the third segment about one and a half times as long as the scape, the fourth segment about one and a half times as long as the scape, not quite one and a third times as long as the third segment, and about two and a quarter times as long as the fifth segment, the rest gradually decreasing to the apex; the segments completely finely and closely punctured, except where the setae arise from rather larger punctures, particularly on the fourth segment where they are very large. The antennal tubercles slightly raised, moderately distinctly; the head moderately and broadly concave between. The frons large, about equilateral, moderately convex, with an extremely fine median longitudinal groove; the lower lobes of the eyes small, narrowing inferiorly, slightly longer than broad, only about two-thirds as long as the genae; the head completely fairly finely and closely punctured, bearing a few long setae anteriorly and at the inner border of the lower lobes of the eyes; finely grayish-tawny pubescent.

The pronotum transverse, convex, with two strongly raised obtuse discal tumescences; with a broadly conical, strong, lateral, almost spinous protuberance on each side slightly post-medially; completely very finely and closely punctured, with a number of irregularly scattered very large punctures in the anterior and posterior transverse grooves, and a few extending, from the anterior groove, posteriorly between the discal tumescences; the transverse grooves very broad and very shallow; dark tawny-brown pubescent in the main, with slight areas of grayish pubescence latero-anteriorly on the posterior border and uniting there, and round the base of the lateral spines, extending to the posterior border. The scutellum sub-triangular, the apex very broadly rounded; very finely and closely punctured; blackish pubescent, narrowly margined with grayish-brown.

The elytra only moderately elongate, not very convex, narrowing a little, but almost straight-sided laterally to about the apical quarter, thence broadly rounded to the apices, which are obliquely truncate, with the marginal angle produced into a strong spine; the centro-basal tumescences moderately strongly raised, strongly black fasciculate; completely finely and closely punctured, with fairly numerous very large punctures scattered here and there, from some of which arise the long setae.

The prosternal protuberance narrow, particularly between the coxae, broadly longitudinally concave medially; strongly curved. The mesosternal protuberance very broad, particularly anteriorly, sub-triangular, truncate apically, the apex slightly emarginate, the truncature about one and a half times as wide as the breadth of the prosternal protuberance medially. The abdominal segments normal; the apical ventrite transverse, more or less broadly rounded, the apex slightly emarginate medially. The underside completely very finely and fairly closely punctured, with a few slightly larger punctures round the apex of the apical segment.

The legs of moderate length; the femora strongly pedunculate; the tarsi slender, the first segment of the posterior about equal in length to the following two united. All the legs very finely and closely punctured; rather sparsely grayish pubescent.

Female.—Similar in color to the male. The antennae slightly shorter, about one and two-

fifths times as long as the body. The apical ventrite more elongate, sub-triangular, the apex truncate and fringed with strong, not very long, hairs.

Length, 6-8.5 mm.; breadth, 2.1-3.2 mm.

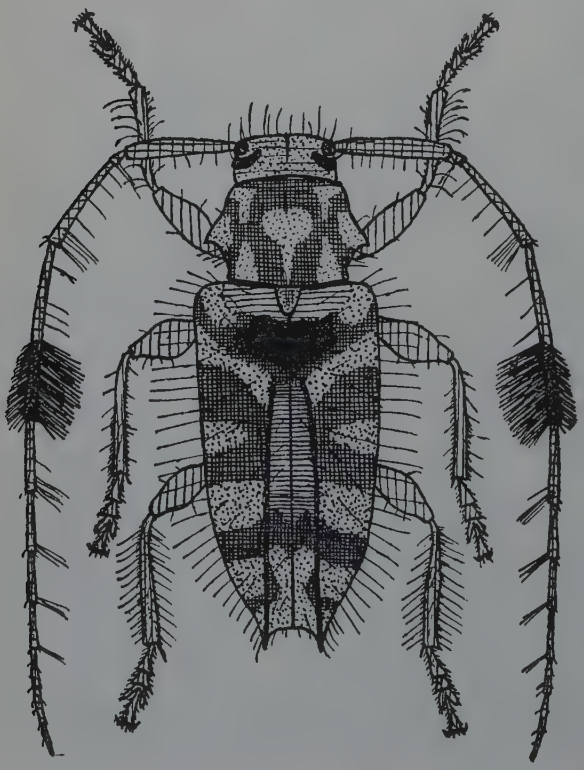
Locality.—Brazil—Santa Catherina (ex. Lacordaire coll.) (Mus. Hist. Nat. Belg.) (Type, ♀); Santa Catherina, Corupa (Hansa Humboldt) (X1.44, A. Maller) (Amer. Mus. Nat. Hist.) (2♀), (1♀ in Gilmour coll. by exch.) id. loc. (X.45, A. Maller) (Amer. Mus. Nat. Hist.) (1♂); (ex. Candeze coll.) (Mus. Hist. Nat. Belg.) (1♂); Rio Grande do sul (Hincks-Dibb coll.) (1♀); Nova Teutonia, (27°11'S, 52°23'W) (Fritz Plaumann) (J. M. Bosq coll.) (1♀); (Amer. Mus. Nat. Hist.) (X-XII, 41) (7♂, 8♀); Nova Teutonia, Corupa (Hansa Humboldt) (J. M. Bosq. in litt.); Est. Sao Paulo (J. M. Bosq in litt.); (Amer. Mus. Nat. Hist.) (X, XII, 44) (1♂, 1♀). Argentina—Misiones (Alta Parana) (J. M. Bosq in litt.).

Material Examined.—Mus. Roy. Hist. Nat. Belg., 2; Amer. Mus. Nat. Hist., 20; Hincks-Dibb collection, 1; J. M. Bosq collection, 1; Gilmour collection, 1. Total 25.

Cosmotoma olivacea sp. nov.

Text-fig. 10

Description. Female.—Dark brown to ferruginous, with olive-green intermixed, particularly on the elytra. The head and pronotum dark brown, the latter becoming ferruginous on the anterior and posterior borders. The scutellum ferruginous. The elytra ferruginous basally between the humeri and suture and extending a little suturally, the rest of the elytra laterally and behind the centro-basal tumescences becoming dark ferruginous. The head covered with thin grayish pubescence; the pronotum covered in the main with dark olivaceous pubescence, variegated with grayish pubescence medially, extending narrowly posteriorly to the base, a small amount antero-laterally and postero-laterally on each side of the disc, and also grayish on the lateral tubercles. The scutellum brownish pubescent, narrowly margined with gray. The elytra thinly ferruginous pubescent basally; for the rest in general dark olivaceous pubescent, broken into fine transverse bands by grayish pubescence as follows: a band from beneath the humeri to the suture just behind the centro-basal tumescences, a broad one from the basal third and one from immediately behind the middle which unite at about the middle of the disc, but do not reach the suture, but end at the suturo-discal obtuse carinae, one at about the apical third, which runs slightly forward to the suture, and finally a very narrow, less distinct, dark olivaceous band at about the apical eighth which reaches the carina, turns anteriorly on the inner side of it and ceases just short of the preceding transverse band; the rest silky variegated gray



Text-fig. 10. *Cosmotoma olivacea* sp. nov. ♀ (× 9.8). Holotype.

pubescent. The underside light ferruginous, covered thinly with grayish pubescence, which is most dense on the sternum and latero-posteriorly on the first and second abdominal segments. The antennal scape greenish; the rest of the segments light ferruginous; very thinly grayish pubescent. The femora light ferruginous basally, the swelling green; the tibiae ferruginous-green; the tarsi ferruginous; all the legs covered sparsely with grayish pubescence.

Ovate-elongate; not very robust. The antennae about one and a half times as long as the body; sparsely fringed below up to the fifth segment; segment two with a thin pencil of setae below, and segments three and five to eight with thin pencils of black setae below at their apices; the fourth segment bearing a large distinct brush of long black setae on about its apical two-fifths, which does not quite surround that apical portion, but leaves about the outer longitudinal quarter naked, the area covered by the setae somewhat swollen; the scape moderately elongate, extending to about the basal third of the pronotum, moderately swollen; the third segment about one and a seventh times as long as the scape, the fourth segment not quite one and two-thirds times as long as the scape, about one and two-thirds times as long as the third segment, and almost two and a half times as long as the fifth segment, the rest gradually decreasing to the apex; the segments completely finely and closely punctured, except where setae arise from rather larger punctures, particularly on the fourth segment where they are much larger. The antennal tubercles slightly raised, the head slightly and broadly concave between. The frons large, about equilateral, moderately strongly convex, with a very fine median longitudinal groove; the lower lobes of the eyes small, narrowing a little inferiorly, about as long as broad, about two-thirds as long as the genae; the head completely finely and closely punctured, bearing a few long setae anteriorly and at the inner border of the lower lobes of the eyes.

The pronotum transverse, very strongly convex discally, the two discal tumescences not separated from one another medially, but forming a large transverse tumescence; with a moderately broadly conical spinous swelling laterally on each side, slightly behind the middle; completely finely and fairly closely punctured, with a somewhat irregular, more or less single, row of very large punctures only on the posterior transverse groove, which is broad and only very slightly broadly concave, the anterior transverse groove not very broad and scarcely at all concave. The scutellum sub-triangular, extremely broadly rounded, almost truncate apically; a little longitudinally concave towards the apex; very finely and closely punctured.

The elytra only moderately elongate, not very strongly convex, almost parallel-sided to about the apical third, thence broadly rounded to the apices, which are obliquely truncate, with the marginal angle produced into a strong spine; the centro-basal tumescences moderately strongly raised, black fasciculate; each elytron with a distinct (because glabrous — probably rubbed), longitudinal, fairly broad, very obtuse, carina running from immediately behind the centro-basal tubercles to the elytral marginal apex, and becoming a little more raised towards the apex; completely finely and closely punctured, with a number of large punctures scattered here and there from some of which arise long setae.

The prosternal protuberance fairly narrow, particularly between the coxae; almost plane and scarcely at all concave; strongly curved. The mesosternal protuberance extremely broad anteriorly, sub-triangular, rather swollen, the sides distinctly bisinuate, towards the apex not immediately truncate, but for a very short but distinct distance becoming parallel-sided, the apex broadly truncate, and very broadly and shallowly emarginate, the truncature about one and a quarter times as wide as the breadth of the prosternal protuberance medially. The abdominal segments of normal size; the apical ventrite transverse, somewhat broadly emarginate; and bearing a distinct median longitudinal groove on its anterior half. The underside completely finely and fairly closely punctured, with a number of distinct larger punctures at the apex of the apical ventrite, which bears short setae.

The legs of moderate length; the femora strongly pedunculate; the tarsi slender, the first segment of the posterior about equal in length to the following two united; all the legs finely and fairly closely punctured.

Male.—Unknown.

Length, 7 mm.; breadth, 2.5 mm.

Locality.—Brazil—Rio de Janiero.

Holotype, ♀, in my collection. Unique.

Diagnosis.—This new species at first glance is very similar to *Cosmotoma viridana* Lacordaire, but on examination is seen to differ distinctly in not possessing a dark brown pubescent transverse post-median band, the two pronotal discal tumescences not separated medially, the pronotum without large punctures anteriorly, etc.

Cosmotoma melzeri sp. nov.

Text-fig. 11

Description. Female.—Dark brown to ferruginous in color, the elytra somewhat dark

TEXT-FIG. 11. *Cosmotoma melzeri* sp. nov. ♀ (× 7.2). Holotype.

olivaceous-ferruginous in part. The head dark ferruginous, the pronotum pitchy-brown in color. The scutellum ferruginous. The elytra somewhat olivaceous-ferruginous in general dermal color, with the apex of the centro-basal tubercles black and black fasciculate, also bearing, at about between the middle and apical two-fifths, a narrow blackish pubescent transverse band, which has its anterior border almost straight, and its posterior border slightly anteriorly curved; also variegated with silvery-gray rather thin pubescence which extends rather narrowly from the humeri round the centro-basal tubercles posteriorly to the suture about midway between the centro-basal tubercles, thence extending rather broadly along the suture to the transverse dark band which it borders anteriorly rather narrowly to the margin; the transverse dark band rather broadly bordered posteriorly with silvery-gray pubescence, and the elytral apex also rather irregularly, chiefly apico-laterally, sparsely grayish. The underside ferruginous covered thinly with grayish-white pubescence, which becomes most dense on the sternum and latero-posteriorly on the first abdominal segment. The antennal scape greenish, the rest of the segments rather dark ferruginous; with thin grayish-brown pubescence. The femora and tibiae greenish, the tarsi ferruginous; the legs covered sparsely with grayish pubescence.

Not very robust, elongate-ovate. Antennae distinctly longer than the body after about the sixth segment, (unfortunately the two apical segments are lacking, but the antennae will be probably a little more than one and a half times as long as the body when complete); rather slender, sparsely fringed beneath up to the fifth segment, on which there are only a few setae and only one or two at the apex of the sixth, the apex of the third segment with a very sparse pencil of hairs beneath, the fourth segment bearing a comparatively small (in this genus) brush of black setae on little more than its apical quarter, which does not completely encircle the segment, only being present beneath and internally in part, the segment a little swollen on the area from which the setae arise. The scape rather elongate and rather slender, not very swollen, extending a little past the middle of the pronotum; the third segment about one and a fifth times as long as the scape, the fourth segment only about one and an eighth times as long as the third, about one and a third times as long as the scape, and almost twice as long as the fifth segment, the rest gradually decreasing to the ninth segment, (thereafter broken, but presumably shorter to the apex); the segments completely very finely and closely punctured, with larger punctures from which the setae arise, particularly on the fourth segment. The antennal tubercles only slightly raised, the head broadly and shallowly concave between; the frons large, about equilateral, moderately strongly convex, with an extremely fine, indistinct, median longitudinal

groove; the lower lobes of the eyes small, distinctly narrowing inferiorly, in fact almost sub-triangular, slightly longer than broad, about equal in length to the genae; the head completely very finely and closely punctured, bearing a few long setae at its anterior border and one or two at the inner margin of the lower lobes of the eyes.

The pronotum not strongly transverse, only about one and a quarter times as broad as long, rather strongly convex, bearing two strongly raised, conical, very obtuse tumescences on the disc; the lateral border rather strongly rounded and swollen anteriorly, running almost regularly to the small lateral spinous conical tubercle, thence rather strongly narrowed posteriorly to the base; completely finely and closely punctured, except for the apices of the discal tumescences, which are impunctate and nitid; with a few sparse very large punctures in the anterior and posterior transverse grooves, these latter being extremely shallow, very broad and almost obsolete. The scutellum sub-triangular, moderately broadly rounded apically; very finely and closely punctured.

The elytra only moderately elongate, only moderately convex, almost parallel-sided to about the apical third, thence broadly rounded to the apices, which are slightly obliquely truncate, with the marginal angle produced into a rather broad, strong, pointed spine; the centro-basal tumescences strongly raised and black fasciculate; completely finely and closely punctured, with large punctures scattered here and there from which long erect setae arise.

The prosternal protuberance moderately narrow, broadly rounded, broadly and slightly longitudinally concave medially. The mesosternal protuberance very broad, somewhat swollen anteriorly, sub-triangular, a little sinuate laterally, the apex broadly truncate, extremely slightly, scarcely at all, broadly emarginate, the truncature about one and a half times as broad as the breadth of the prosternal protuberance medially. The abdominal segments of normal size; the apical segment transverse, the apex very broadly truncate, very slightly roundly so; the lateral angles rounded, with a number of large distinct hair-bearing punctures towards apex, (the abdomen is somewhat ventrally deflexed so that the anterior half of the apical ventrite is not visible and the groove on the anterior half, which I think in this species must be very feeble, is not visible; the strong setae-bearing apical punctation is, however, normally a female character in this genus). The underside otherwise completely very finely and rather variably closely punctured.

The legs of moderate length; the femora pedunculate; the tarsi moderately slender, the first segment of the posterior tarsi about equal in length to the following two united; all the legs finely and closely punctured, with a few slightly larger punctures scattered here and there.

Male.—Unknown.

Length, 8.5 mm.; breadth, 2.9 mm.

Locality.—Brazil—Bahia.

Holotype, ♀, in my collection. Unique.

Diagnosis.—This distinct new species differs from all the other known species of the genus in the much smaller size of the brush of setae on the fourth antennal segment and in the rather different pronotal shape, which is more rounded laterally, etc. From *Cosmotoma viridana* Lacordaire, it differs further in the almost straight, narrow, dark-colored, post-median elytral band, in being distinctly brownish in appearance and not greenish, as well as other distinct characters.

Cosmotoma nigra sp. nov.

Text-fig. 12

Description. Male.—Completely pitchy-black above and below, without any markings of any kind; the base of antennal segments five to eleven pale ferruginous-yellow annulate on about their basal quarter to third; completely covered, sparsely and extremely finely, with short grayish pubescence, which is nowhere dense enough to give a grayish appearance, except perhaps where a little denser on the upper surface of the intermediate and posterior femora.

Not very robust, somewhat ovate-elongate in shape, but the elytra somewhat attenuate apically. The antennae about one and a half times as long as the body, sparsely fringed below up to about the sixth segment, segments three and five to seven with thin pencils of setae beneath their apices; the fourth segment bearing a large distinct brush of black setae on about its apical half, which does not completely encircle the segment, but only about the inner longitudinal half, the area bearing the setae distinctly swollen; the scape moderately elongate, extending to about the basal quarter of the pronotum, moderately swollen; the third segment very slightly shorter than the scape, the fourth segment about one and a sixth times as long as the scape, almost one and a third times as long as the third segment, and about one and three-quarter times as long as the fifth, the rest gradually decreasing to the apex; the segments completely finely and closely punctured, except where the setae arise from larger punctures. The antennal tubercles only very slightly raised, the head slightly and broadly concave between. The frons large, very slightly transverse, moderately convex, with a fine median longitudinal groove; the lower lobes of the eyes small, only very slightly narrowing inferiorly, almost sub-quadrate about three-

TEXT-FIG. 12. *Cosmotoma nigra* sp. nov. ♂ (× 10.7). Holotype.

quarters as long as the genae; the head completely finely and closely punctured, bearing only very few long setae anteriorly and at the inner margin of the lower lobes of the eyes.

The pronotum transverse, moderately convex, bearing two strong discal tumescences; with a strong, broad, conical, obtuse swelling laterally on each side, slightly post-medially; completely very finely and closely punctured, with a few sparse very large punctures scattered in the anterior and posterior transverse grooves, and few anteriorly between the discal tubercles. The transverse grooves broad, the posterior very shallow, the anterior more distinct. The scutellum sub-triangular, moderately broadly rounded apically; finely and closely punctured.

The elytra only moderately elongate, not strongly convex, narrowing towards the apices, which are a little obliquely truncate, with the marginal angle strongly spinously produced; the centro-basal tumescences moderately strongly raised and densely black fasciculate; completely very finely and fairly closely punctured, with larger punctures scattered here and there from which erect setae arise.

The prosternal protuberance narrow, particularly between the coxae, slightly longitudinally concave, moderately strongly curved. The mesosternal protuberance very broad and rather swollen anteriorly, sub-triangular, the apex truncate and very slightly emarginate, the truncature a little wider than the breadth of the prosternal protuberance medially. The abdominal segments of normal size; the apical ventrite transverse, somewhat broadly rounded, its apex rather broadly emarginate. The underside completely finely and fairly closely punctured, with a few slightly larger punctures on the sides of the abdominal segments.

The legs of moderate length; the femora pedunculate; the tarsi slender, the first segment of the posterior tarsi very slightly longer than the following two united; all the legs fairly finely and fairly closely punctured, with a number of slightly larger punctures scattered here and there.

Length, 7 mm.; breadth, 2.5 mm.

Locality.—Brazil—Santa Catherina.

Holotype, ♂, in the Musée Royale d'Histoire Naturelle de Belgique. Unique.

Diagnosis.—This new species is conspicuously different from *Cosmotoma adjuncta* Thomson and all other known species in the genus in being uniformly black in color.

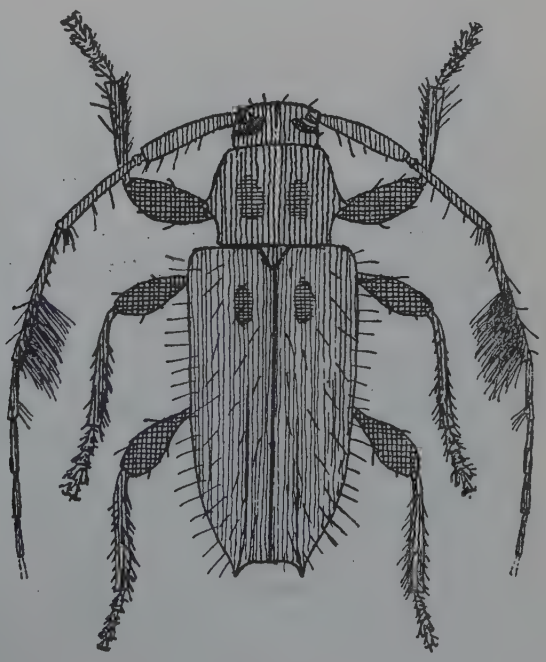
COSMOTOMA PALLIDA sp. nov.
Text-fig. 13

Description. Female.—In general appearance uniform yellowish-brown. The dermal color of head, pronotum and underside dark pitchy-brown, of the elytra fulvous-ferruginous; the antennae, femora and tibiae greenish to greenish-ferruginous, the tarsi light ferruginous, completely covered with very thin, short, fulvous pubescence, without any trace of other color except extremely slightly darker suturally at about the apical third, and the black fasciculae.

Ovate-elongate; not very robust. The antennae distinctly longer than the body (unfortunately broken after the eighth segment in the best specimen, but probably about one and a third times as long as the body when complete); very sparsely fringed below up to the fifth segment, the third and fifth and perhaps the sixth segment bearing thin pencils of setae beneath; the fourth segment bearing a large distinct brush of black setae on about its apical two-fifths, which does not completely encircle the apical area, but leaves about the outer longitudinal half naked, the area bearing the setae distinctly swollen; the scape moderately elongate, extending to about the basal third of the pronotum, not very strongly swollen; the third segment about equal in length to the scape, the fourth segment about one and a third times as long as the scape, about two and a third times as long as the fifth segment, the rest gradually decreasing to the apex; the segments completely finely and closely punctured with larger punctures from which the setae arise. The antennal tubercles only a little raised, the head broadly concave between. The frons large, about equilateral, moderately strongly convex, with a very fine median longitudinal groove; the lower lobes of the eyes small, narrowing inferiorly, about one and a quarter times as long as broad, about two-thirds as long as the genae; the head completely finely and closely punctured, with only a very few extremely sparse setae at the lower border and inner borders of the lower lobes of the eyes.

The pronotum transverse, moderately convex, bearing two strong discal tumescences; with a moderately strong spinous swelling laterally on each side, slightly behind the middle; completely very finely and closely punctured, with a more or less single irregular row of very large punctures in the anterior and posterior transverse grooves, which are broad and not very strongly concave, also a few of these large punctures antero-medially almost between the two discal tubercles. The scutellum sub-triangular, a little elongate, rather narrowly rounded apically; very finely and closely punctured.

The elytra only moderately elongate, not very strongly convex, more or less parallel-sided to about the apical third, thence broadly rounded to the apices, which are obliquely truncate, with the marginal angle produced into a strong sharp pointed spine; the centro-basal tumescences moderately strongly raised and black fasciculate;



TEXT-FIG. 13. *Cosmotoma pallida* sp. nov. ♀ (× 8.0). Holotype.

completely very finely and closely punctured, with larger punctures scattered here and there, from some of which long erect setae arise.

The prosternal protuberance narrow, particularly between the coxae, where it is rather parallel-sided for a short distance; rather strongly curved. The mesosternal protuberance very broad and rather swollen anteriorly, sub-triangular, the apex truncate and slightly, but distinctly, emarginate, the truncature only slightly wider than the breadth of the prosternal protuberance medially. The abdominal segments of normal size; the apical transverse, but a little conical, the apex very broadly truncate, bearing a distinct median longitudinal groove on its anterior half. The underside completely finely and fairly closely punctured, with numerous large, setae-bearing punctures at the apex of the apical ventrite.

The legs of moderate length; the femora pedunculate; the tarsi slender, the first segment of the posterior tarsi about equal in length to the following two united; all the legs finely and moderately closely punctured, with a few slightly larger scattered punctures.

Male.—Quite similar in color to the female. A little less robust, and the elytra less parallel-sided, more attenuate to the apices.

The apical ventrite transverse, less elongate than the female, more or less broadly rounded, the apex slightly, but distinctly emarginate, the lateral angles rounded; lacking, or with very many fewer, large seta-bearing punctures.

(The comparative antennal length unknown as the antennae are completely lacking in the only male specimen examined).

Length, 7.5-8.5 mm.; breadth, 2.8-3.2 mm.

Locality—Brazil—Santa Catherina (1♂, 2♀).

Holotype, ♀, Allotype, ♂, in my collection. Paratype, ♀, (from my collection No. 6284) in the United States National Museum. (Exchanged for the paratype of *Cosmotoma fasciata* Fisher).

I have made the female the Holotype because two almost complete females were seen, the male lacking its antenna.

Diagnosis.—This new species is conspicuously different from *Cosmotoma adjuncta* Thomson and all the other known species in the genus in being uniformly fulvous-yellow in color.

Cosmotomella gen. nov.

Description.—Moderately elongate, sub-parallel, finely pubescent, silky or with silky reflections, with erect setae throughout.

Head large, concave between the antennal tubercles; frons transverse; eyes small, finely granulated; genae elongate. Antennae of male about one and a half times as long as the body, in the female about as long as or slightly longer than the body; with erect hairs beneath; scape slender, slightly longer than the third segment, fourth segment longer than the third, the fifth to eleventh gradually decreasing; fourth segment bearing beneath a fascicule of long hairs. Pronotum about as long as broad, sub-globose, strongly convex dorsally, the disc bearing two feeble tubercles; broadly rounded, but only feebly tuberculate, not spinous laterally. Scutellum sub-triangular in male, broadly rounded in female. Elytra straight in front, scarcely broader than the pronotum at widest; moderately elongate, rather straight-sided, sub-parallel laterally, narrowing gradually to the apices which are sinuately truncate, the marginal angles spinous; a strong centro-basal tumescence. Legs of moderate length; setose; femora pedunculate, very swollen distally; tarsi rather short, the first segment of the posterior about as long as segments two and three united. Prosternal protuberance not very broad, curved posteriorly; mesosternal protuberance broad, triangular. Apical ventrite broadly rounded and emarginate apically in male, a little more elongate and broadly rounded apically in female, the last segment in female with a distinct median longitudinal groove on the basal half.

Genotype: Cosmotomella zikani Melzer, 1927. Brazil.

This new genus, created for the reception of the species described as *Cosmotoma zikani* Melzer, differs conspicuously from *Cosmotoma* Blanchard in lacking lateral pronotal spines, in the centro-basal elytral crest not bearing a fascicule of hairs, in the pronotal shape varying between the two sexes, and the more elongate and parallel form.

Table 1 gives the relative proportions of length to breadth of *Cosmotomella zikani* Melzer and the species of *Cosmotoma* Blanchard. From this it will be seen that *Cosmotomella zikani* Melzer is always slightly more than three times as long (in total length) as broad, whereas all the species of *Cosmotoma* Blanchard are less than three times. Also the elytral length of *Cosmotomella zikani* Melzer is always slightly more than twice the breadth, whereas in the species of *Cosmotoma* Blanchard it is less than twice.

Cosmotomella zikani Melzer

Text-figs. 14 ♀, 15 ♂

Melzer, 1927, Rev. Mus. Paulista, *15*, 574.

A pair of specimens in my collection agree with Melzer's description almost completely, except in one or two, perhaps minor, points. Examination of these during the course of this revision showed further sexual dimorphism than

TABLE 1. COMPARISON OF BODY PROPORTIONS BETWEEN THE SPECIES OF THE GENERA *Cosmotoma* SERVILLE AND *Cosmotomella* GILMOUR.

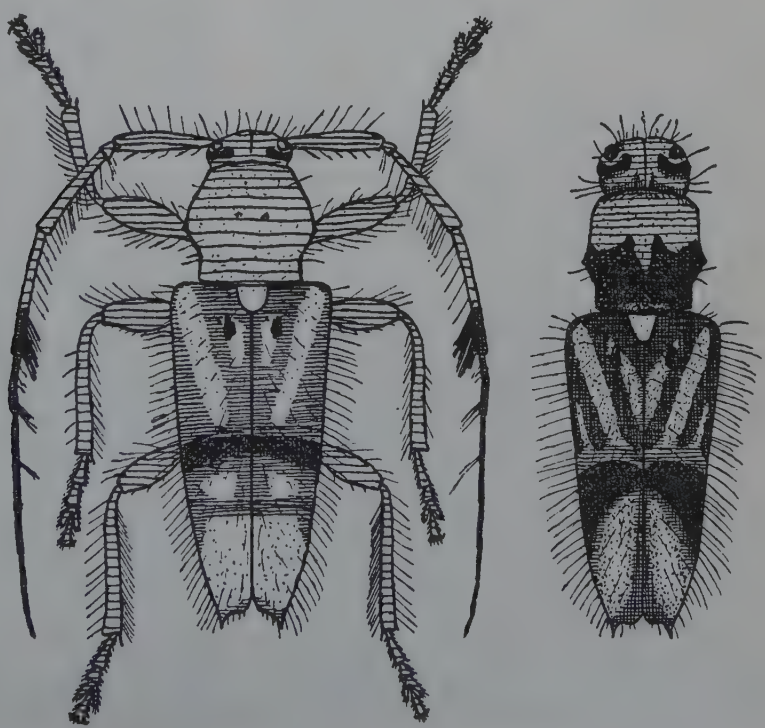
Species	Body: Breadth (1) (Averages)	Elytra: Breadth (1)
COSMOTOMA		
olivacea Gilmour	2.58	1.79
sertifer Serville	2.77	1.95
nigra Gilmour	2.65	1.73
suturalis Gilmour	2.76	1.96
adjuncta Thomson	2.69	1.75
rubella Bates	2.69	1.79
nigricollis Bates	2.64	1.72
melzeri Gilmour	2.90	1.85
pallida Gilmour	2.73	1.91
triangularis Gilmour	2.59	1.74
viridana Lacordaire	2.82	1.81
COSMOTOMELLA		
zikani Melzer	3.14-3.3	2.14-2.15

noted by Melzer, and further differentiating features from *Cosmotoma* Blanchard, in which Melzer originally placed the species, while noting that it did not completely agree with the generic characters of this genus. I have, therefore, as described above, created a new genus, *Cosmotomella,* for the species *zikani* Melzer.

Comparison is not possible between the male antennae, for in my specimen these are lacking after the scape. Further, Melzer states that the pronotum is unarmed laterally. I cannot believe that such an apparently competent observer as Melzer would overlook any trace of tubercle and must therefore point out that while there is only an extremely faint trace in my female specimen, in the male it is visible, though feeble, and somewhat more superior than in the genus *Cosmotoma* Blanchard. Perhaps it is possible that in *zikani* Melzer there is an individual variation in pronotal shape. Melzer does not comment on any undue sexual difference in pronotal shape, but in my two specimens this is quite conspicuous, as will be seen by the figures, and yet I am quite sure that my specimens are the same species, showing also a similar sexual color difference as noted by Melzer.

In view of these differences I give, firstly a translation of Melzer's original description, so that other workers may draw their own conclusions.

"Related to C. *viridana* Lacord., olivaceous-piceous, clothed with silky silvery pubescence, interspersed with long erect setae, the elytra marked with a black fascia behind the middle; the head large, concave between the antennae, the frons transverse, sub-planate, the eyes small,



TEXT-FIG. 14 (Left). *Cosmotomella zikani* Melzer. ♀ (× 7.0).

TEXT-FIG. 15 (Right). *Cosmotomella zikani* Melzer. ♂ (× 7.9).

minutely granulated, the genae elongate; the antennae of the ♂ a half longer than the body, in the ♀ over-reaching the apex of the elytra by the last three segments, the scape slender, slightly shorter than the 3rd segment, the 4th longer than the preceding, 5-11 sub-equal, elongately fimbriate beneath, the 4th segment ornated beneath the apex with a crest of hairs; the thorax not broader than long, sub-globose, the base strongly constricted, strongly convex dorsally and armed with two obsolete tubercles placed transversely in the middle, unarmed laterally, the scutellum densely silky-silvery pubescent; the elytra almost equal to the thorax at the greatest width, straight truncate basally, gradually attenuate posteriorly, the apices themselves singly sinuately truncate, the external angles spinose, the centro-basal carinae strong, not fasciculate, not costate posteriorly; the legs subequal, the femora pedunculate, strongly clavate; the prosternal process moderately broad, the mesosternal process broad; the metasternum rather densely silky pubescent, the mesosternal and metasternal epimera and also the posterior margin of the 1st abdominal segment with white pubescence.

"Length, 8.25-10 mm. 1♂, 2♀.

"*Locality*.—Fazenda Jerusalem, Estado do Espirito Santo, Rio Muriahé, Estado do Rio de Janiero, Rio José Pedro, Estado de Minas Geraes, J. F. Zikan leg.

"Through its principal characters, this longicorn appertains to the genus *Cosmotoma*, but because of the complete lack of the lateral spines of the prothorax, etc., fails in similarity.

"Through the more parallel form, through the lack of the spine on each side of the prothorax, through the lack of the fascicules hairs and of costae on the elytra and further through the fascicules much reduced on the fourth antennal segment, this species easily distinguishes itself from *C. viridana* Lacord.

"The transverse band on the elytra in the ♀ is narrow and opaque, in the ♂, however, it is much broader, lustrous and accompanied through to the suture as if forming a bridge." (Translation of the original description.)

Male.—Head and pronotum black; elytra pitchy to dark ferruginous; antennae, legs and underside ferruginous. Head and pronotum with variably dense olivaceous pubescence; on the head most dense on the genae, between the antennal tubercles and round the eyes; on the pronotum most dense on the anterior half and medially, on the minute lateral tubercle whitish. The scutellum densely white pubescent. The elytra with, in general, dark brown pubescence, marked with olivaceous and white pubescence; the dark brown pubescence becoming very dense and forming a somewhat curved post-median complete fascia which distinctly broadens to the margin; the olivaceous pubescence as follows: along the anterior border of the fascia, a vitta from the humeri to the fascia near the suture, and suturally on the anterior half; the white pubescence as follows: a narrow short pre-median vitta at the side of the disc, and a broad oblique irregular area on the posterior half, which gradually becomes olivaceous round its borders. The antennae and legs finely olive-gray pubescent, on the latter becoming white on the tibiae and tarsi. The underside finely olivaceous pubescence; the mesosternal and metasternal epimera, and the postero-lateral border of the first abdominal segment with dense white pubescence.

The antennal scape slender, rather elongate, extending to about the basal third of the pronotum; finely and closely punctured; sparsely but distinctly fringed beneath; (all the other segments unfortunately lacking in the only male examined). The antennal tubercles moderately raised, widely separated. The frons very slightly transverse, moderately but distinctly convex; the lower lobes of the eyes small, narrowing inferiorly, about as long as broad, about two-thirds as long as the genae; the head completely finely and closely punctured, with numerous long, erect setae.

The pronotum about as broad as long, subglobose, only slightly rounded laterally to about the middle, thence distinctly constricted to the base; bearing laterally, slightly behind the middle on each side a small obtuse tubercle; the disc strongly convex, bearing medially on each side a distinct broad, obtuse tubercle; the whole very finely and fairly closely punctured, much more sparsely on the discal tubercles, and with two or three large punctures anteriorly and a few posteriorly near the borders. The scutellum sub-triangular, rounded apically; about as long as broad; very finely and closely punctured.

The elytra elongate, attenuate to the apices; a little broader basally than the pronotum medially; the humeri distinct; rather convex, but with a distinct elongate depression from the inner side of the humeri; somewhat abruptly declivous at about the apical fifth; the apices each distinctly, slightly sinuate emarginate, the marginal angle strongly spinous, the sutural angle rather narrowly rounded; each elytron with a strongly raised centro-basal tumescence; the whole very finely and closely punctured, with a few very large punctures on the disc just behind the basal tumescences; with numerous long erect setae.

The prosternal protuberance moderately broad, shallowly, longitudinally depressed me-

dially; moderately strongly curved. The mesosternal protuberance very broad basally, broadly sub-triangular, the apex truncate, the truncature about as broad as the prosternal protuberance medially, somewhat plane above, gradually rounded anteriorly. The abdominal segments of normal size; the apical ventrite slightly transverse, rounded apically, with a very shallow, obtuse, median emargination. The underside more or less completely finely and closely punctured, the punctures more sparse on the abdominal segments, the apical ventrite a little coarsely punctured, particularly towards the apex.

The legs of moderate length, (comparatively a little more elongate than in the genus *Cosmotoma*); the femora pedunculate, strongly clavate; the tarsi rather elongate and rather slender, the anterior the broadest; the first segment of the posterior tarsi about equal in length to the following two segments united; the tarsal claws divaricate. All the legs very finely and closely punctured; with numerous long erect setae, particularly on the tibiae, which are also most densely pubescent.

Female.—More robust than the male, and of moderately similar aspect, but differing conspicuously in many ways on examination.

The general color lighter. The head and pronotum more or less uniformly and densely olivaceous pubescent; this is a little yellowish on the middle of the head and middle of the pronotum. The scutellum similarly densely white pubescent. The elytral general brown pubescence somewhat lighter; the dark brown transverse fascia just behind the middle narrower, almost straight, and not widening laterally; the olivaceous pubescence as found in the male, more grayish-olivaceous and a little more extensive, the short white lateral premedian vitta more or less lacking, the apical third rather more extensively silky grayish to olivaceous-gray pubescent. The legs more densely grayish pubescent. The antennae grayish pubescent, becoming light brown from the apical third of the fourth segment. The underside more olivaceous-yellow pubescent; the metasternum almost completely grayish-yellow pubescent; the white epimera of the mesosternum, metasternum and first abdominal segment somewhat less distinct owing to the more dense general pubescence.

The antennae about one and a sixth times as long as the body; slender; fringed below, although only sparsely and apically on segments after the fourth; the fourth segment bearing a moderate brush of setae below on its apical third; the scape slender, moderately elongate; the third segment about equal in length to the scape, the fourth segment about one and a third times as long as the scape; the following segments gradually decreasing to the apex, the fifth segment slightly curved; the segments completely finely and closely punctured. The frons slightly transverse, much less convex than in the male, and the median longitudinal groove more distinct.

The pronotum much more distinctly globose than in the male; the lateral tubercle almost obsolete, scarcely discernible; the two discal tumescences much smaller and less distinct, the general convexity being stronger. The scutellum larger, about as long as broad, not sub-triangular, more or less regularly rounded.

The elytra slightly less attenuate than in the male, comparatively not so much broader than the pronotum; otherwise structurally similar to the male; the centro-basal tumescences with a few long erect setae, (these are only present on the left elytron, so it is possible that they were originally present in the male; they do not form a fascicule); the lateral apical elytral spine a little broader and not quite as elongate.

The underside similar to that of the male, except, the apical truncature of the mesosternal protuberance broader, about one and a half times as broad as the prosternal protuberance medially; the apical ventrite comparatively a little more elongately conical, bearing a very distinct median longitudinal groove on its anterior half, the apex broadly rounded and with a number of very large coarse punctures and short setae.

The legs a little less elongate compared to the male. Tarsal proportions similar to those of the male.

Length, 8.5-9.5 mm.; breadth, 2.9-3.1 mm.

Locality.—Brazil: Espirito Santo (1♂); Rio de Janiero (1♀).

Material Examined.—Gilmour collection, 2.

2

Two Little-known Selective Insect Attractants[1]

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(Plates I-IV)

[This paper is one of a series emanating from the tropical Field Station of the New York Zoological Society, at Simla, Arima Valley, Trinidad, British West Indies. This station was founded in 1950 by the Zoological Society's Department of Tropical Research, under the direction of Dr. William Beebe. It comprises 200 acres in the middle of the Northern Range, which includes large stretches of undisturbed government forest reserves. The laboratory of the station is intended for research in tropical ecology and in animal behavior. The altitude of the research area is 500 to 1,800 feet, with an annual rainfall of more than 100 inches.

[For further ecological details of meteorology and biotic zones see "Introduction to the Ecology of the Arima Valley, Trinidad, B.W.I.," William Beebe. (Zoologica, 1952, Vol. 37, No. 13, pp. 157-184).]

CONTENTS

I. INTRODUCTION

INSECT repellents have earned a generous representation in entomological study and literature, owing perhaps to their economic importance.

Attractants appertain rather to pure science and are more directly related to our studies of animal behavior. They are abundant in variety and varied in efficiency and, at the Zoological Society's station at Simla, Trinidad, B.W.I., are brought to our attention the moment we leave the laboratory. This will be evident from a few random examples.

Under odor we may mention tree-bait, with the old familiar mixture of such ingredients as molasses and beer, attractive more especially to moths at night; sexual odors, as the well-known drawing of males to a caged female from as far away as two to six miles; plant odors, operating sometimes indirectly, as when an adult butterfly oviposits on a larval food-plant. Finally, we have the olfactory focus of over-ripe fruit or decaying mouse which influences a whole complex of eager receptors.

Light is one of the best-known attractors, as indicated by the swarms around an electric light globe or reflected light from a sheet. The power of color is shown when an iridescent morpho butterfly swoops down upon a bit of blue paper. The auditory attractant of a cicada is clearly evident to our senses, and the exact tone of the wings of a female mosquito is an attractant to her mate.

More mysterious than any of the foregoing is the impelling force of mass migration, a yielding to an instinct which, at least basically, is inexplicable. At this moment, thousands upon thousands of insects of almost all orders, are pouring southward through a sixty-foot wide pass near Rancho Grande, Venezuela, repelled from their place of birth or attracted to an unknown area by some all-inclusive impulse.

The chief object of this casual and heterogeneous list of attractants is to point out that certain ones are selective, carrying their messages to individual species, while the remainder are more or less general, appealing to a wider range of recipients.

[1] Contribution No. 952, Department of Tropical Research, New York Zoological Society.

II. Fedegoso or Wild Heliotrope Association

Early in the occupancy of the station at Simla, we selected the family of moths, Euchromidae, for particular study. Among the considerations which prompted this were intricate instincts of the larvae, day-flying habits of many of the adults and the frequency of apparent mimicry.

During the first season, from December, 1952 to May, 1953, our collecting was restricted to three methods: pursuit, in the field, of free-flying moths; the capture of those which alighted on the laboratory screens in the daytime; and capture of the nocturnal forms that came to an illuminated sheet.

All this was revolutionized the following season by the use of the common weed, *Heliotropium indicum* Linnaeus, which proved to be a remarkably efficient and selective attractant. Our attention was directed to this phenomenon by the notes of G. Hagmann (1938) and A. Miles Moss (1947).

Our cultivated garden heliotrope is derived from the South American wild species, *Heliotropium peruvianum* Linnaeus, and is characterized by the clustered appearance of the blossoms, and their strong, sweet, vanilla-like scent.

The Indian heliotrope, *Heliotropium indicum,* was named by Linnaeus 202 years ago. It has spread from its native home in Asia, and has been acclimated in the New World to such a degree that its present distribution extends from Virginia and Illinois south through Central and South America to Buenos Aires. It has received many common names, the one we have chosen being "fedegoso," although this introduces a semantic misunderstanding. This is a Portuguese word meaning ill-smelling, whereas Hagmann characterizes it as "exquisite." To our senses the dry foliage of the heliotrope gives forth a not-unpleasant, somewhat pungent, musty smell, such as might distinguish a long-used herbarium. Other names, such as eyebright, refer to alleged curative properties. Still other terms, cocks-comb and scorpion plant, relate to the shape of the flower spike.

Shortly after our return to Simla, on December 24, 1953, Research Assistant Rosemary Kenedy located a plant of the fedegoso with the assistance of the Botany Department of the Imperial College of Tropical Agriculture. From then on, assiduous search revealed many scattered clumps of this plant. It grows in waste places, such as old, neglected gardens and fields, usually singly or in small clumps. It is a typical weed, wholly undistinguished, without intensive odor or color. This wild heliotrope is a small plant, from one to four feet in height, with single, curved spikes of small, pale lilac blossoms. These spikes or racemes are often divided longitudinally into thirds; the terminal third with unopened buds, the middle of full-blown flowers, and the basal third of developing seeds. The stems are hairy, the branches coarse, the leaves large, and oval or ovate. The roots are short and thick and have only a comparatively slight hold on the soil.

In our use of the weed, uprooting is the first step. A half dozen plants are pulled up and shaken free of soil. At Simla the roots are tied together with twine and the cluster is suspended upside-down from some low branch or from a stake driven into the ground. A favorite place is along an open trail through the jungle or in an area free of vegetation close to the forest's edge. The leaves shrivel soon after the plant is collected and lose whatever of apparent symmetry or character they may have possessed. During subsequent days of sun and rain the foliage becomes in succession dry and brittle, saturated and sodden.

For the first two or three days little activity is observed around the withered plants. Then one, two, a dozen butterflies and moths appear, coming upwind, and all alight. The desiccated racemes of flowers and seeds seem to exert especial attraction, but the stems, leaves and roots are far from neglected.

The dominating point of interest in fedegoso, as an attractant, is its selective quality. In the course of five months of observation in and around Simla, we detected members of only four families of Lepidoptera coming to the bunches of dead plants. Two of these were butterflies, Danaidae and Ithomiidae, and two were moths, Euchromidae and Arctiidae. Other groups, such as heliconids, nymphalids and pierids, sometimes flew past the dry vegetation, but no individual ever alighted or even hesitated. Added to this is the fact that in most classifications the four selected families are placed at the top of their respective groups, presumably indicating extreme specialization in the scale of lepidopteran phylogeny.

This remarkable, selective, attractant phenomenon was reaffirmed during a few weeks of our experience with fedegoso in Surinam, and in reports from Pará, of Hagmann and Moss.

The selective quality of fedegoso is apparent in other than lepidopteran families. Details of specific selection as shown in Euchromidae will be discussed in future papers. One example will suffice here. Until we began to use this method of collecting we had never come across a specimen of *Sphecosoma trinitatis* Rothschild, a close mimic of a small *Polybia* wasp. The resemblance

is so exact that only close examination reveals the difference between moth and wasp. In the course of five months we took or observed one hundred and forty-seven of these wasp-mimicking moths on fedegoso, and these resolved into three distinct species, two of which had not heretofore been recorded from Trinidad.

Arctiidae was the only other family of moths attracted by fedegoso, and this sparsely, as only fourteen specimens of seven species were taken, three of which were uniques.

As euchromids were the dominant fedegoso group among moths, so ithomiids were, far and away, the more numerous of the butterflies. Fifteen species of Ithomiidae have been recorded from Trinidad. Of these, on March 21, 1954, at 8 A.M., on two adjacent bunches of fedegoso, I observed or collected eight species, totalling a minimum of 257 individuals. The shade-loving, skeleton-winged species *Ithomia drymo pellucida* Weymer and *Hymenitis andromica trifenestra* (Fox) excelled in numbers, comprising three-fourths of the total ithomiids.

We recorded small numbers of two species of Danaidae, the first of which, *Danaus plexippus megalippe* (Hübner), is the tropical representative of our northern Monarch. The striking *Lycorea ceres ceres* (Cramer) was present in numbers and the same individuals often returned day after day, conspicuous in their ithomiid type of pattern and coloring.

There seems little doubt that the first appeal of the attraction of fedegoso is through the olfactory senses of the Lepidoptera, as evinced by the approach upwind, together with the total lack of advertising or directive coloring in the plant.

Soon after the insect alights, or occasionally just before, the tongue unrolls, the tip probing and prodding about as the organ comes into function. Careful examination of the surface tissues of fedegoso fails to reveal any evidence of liquid drops or other source of nourishment. Nevertheless, so potent is some such aliment that it affects the whole behavior of the insects. After a short period of feeding the insect loses its timidity and will often permit itself to be picked up by the wings, examined and replaced, or it may be captured by gently slipping a glass vial over it. When crowds of ithomiids are clustered close together, they will often buffet one another without taking flight.

During this first season nothing was done about chemical or other study of the nutritive substance of fedegoso. While it exerts a noticeable effect on the reduction of the escape reaction, this is in no sense through what might be called intoxication, as in the case of butterflies feeding on fermenting fruit. The insects show an obvious reluctance to leave their repast, but once on the wing, their flight is swift, accurate and typical. They quickly return to their feeding on leaf or raceme.

A quotation from the notes on fedegoso in Pará, Brazil, by A. Miles Moss will present the similarities and the differences of our observations on the same plant. The suspended, dried plants, he says, "constitute a most remarkable attraction for the great majority, though not all, of the species of Syntomidae [Euchromidae], as well as for the closely related Arctiidae; also for many Danaiid butterflies, particularly the Ithomiinae, for wasps of many kinds, for a few beetles, for grasshoppers, for bugs, mosquitoes and flies of all sorts." Aside from Lepidoptera, there is considerable discrepancy between recorded visitors as observed at Pará and at Simla. At the former place in Brazil numerous non-lepidopteran visitors were observed. In our experience, such insects are rare or absent. In the case of mosquitoes, Hagmann indicates that they divide their interest between fedegoso and the observer, who is attacked "unmercifully."

Of the non-lepidopterans we noted only three species of wasps, which occurred rarely, and on plants desiccated for more than two weeks, so old that they had lost their attractiveness for moths and butterflies. Besides this, there were two species of small longicorns which, on four occasions, were found, usually in pairs, wandering about the dry foliage. Now and then we had hints of minor adaptations and inter-relationships beginning to take shape on our fedegoso. Twice we found flower spiders and once an orb-weaver trapping and devouring unwary euchromids. On another occasion a mantid had found the dry vegetation good hunting and was holding a euchromid in the grip of its forelegs, and a ponerine ant was discovered carrying off an ithomiid. The several times that we discovered butterflies sleeping on the same twig from which they had been feeding during the day suggested opportunities for new arthropod relationships.

Heliotropium is a genus of plants of the borage family, Boraginaceae, and the thought occurred to us that botanical consanguinity might carry with it some of this mysterious attraction for insects. Miss Kenedy made a few preliminary experiments with shrivelled twigs and leaves of two species of the genus *Cordia,* namely, *C. alliodora* or Cypre, and *C. cylindrostachya* or Black Sage. These gave no positive results.

Of a third genus of the borage family, *Tournefortia,* we had no available material. Upon our return to New York we received a letter from Dr. P. A. Buxton referring us to notes made by himself, 1926, and by Mr. G. H. E.

Hopkins (1927) on trees of this genus growing on Samoa and other Pacific islands. Dr. Buxton writes that he found that "males of the genus *Euploea* of several species, in several different islands in the South Pacific, occur in numbers on withered twigs of the tree *Tournefortia*. The insect does not feed on that tree and the butterflies do not visit the flowers of that tree. The observations were made repeatedly but one cannot explain them."

This gives hope that more extensive experimentation with members of the borage family may reveal other related plants that share this attractant quality.

III. Tangerine Association

On March 6 and on two successive days I observed a Cacao Caligo, *Caligo eurilochus minor* Kaye, resting on a particular spot on the branch of a tangerine tree, a citrus, *Citrus nobilis* Andre, whose fruit is otherwise known as portugal or mandarin orange. The tree was one of a row in the citrus grove on Water Trail, a few yards to the east of Simla Laboratory. The insect was unusually fearless and permitted close observation. It was busily probing with its tongue in a small area of what looked like the exudate of a spittle insect.

Subsequent observation and consultation with Dr. Egbert Tai of the Government Agricultural Experiment Station tentatively identified the puddle of white foam as probable evidence of the Crotch Disease of tangerines. Of this malady it is said that the cause is not known but a virus is suspected. One of the most conspicuous symptoms is a white froth, which may ferment, and oozes from openings in the bark. From time to time there may be a rehealing. Examined under a hand lens, bubbles are seen to surge up from below, burst, and their place taken by others. Except for these little scattered lesions the disease seemed no detriment to either blossoms, fruit or foliage of the trees under observation.

On the branches of two adjacent tangerine trees there were several small areas of the pale exudation. These seemed to bubble and to overflow at times, and again in temporary drought they dried up. At all times they were sources of intensive attraction to certain insects. As in fedegoso, the attractive agent was strongly and definitely selective.

A. Lepidoptera

In the course of several weeks of intermittent watching I observed fifteen species of butterflies alighting at one or the other of three small areas of bubbling froth, small puddles not more than one-half by one inch. I bored holes through the bark and wood, and daubed sugar and honey on the trunks, but failed to distract the attention of the insects from their chosen ambrosia. Seldom did a butterfly alight on the trees except within tongue's reach of the froth. On the occasions when this did happen, there was instant approach to the attractive substance.

The fifteen butterflies were distributed among three related families, Nymphalidae, Morphidae and Brassolidae, in the proportion, of known Simla species, of 33%, 100%, and 60%. Although few in number of species, this group nevertheless included the largest and, in color and pattern, the most striking of Trinidad's tropical butterflies.

The following is a list of the species of Lepidoptera observed at the tangerine feeding lesions, together with a few casual notes.

The large, Green-checkered Nymphalid, *Victorina steneles steneles* (Linnaeus), joined the tangerine association three times. One marked individual returned repeatedly for a week. When feeding it kept its wings closed, not flattened in the normal position of sunning. Also it confined its visits to the sunniest, warmest parts of the day.

Peridromia arethusa (Cramer), the Blue-spotted Black, visited the trees day after day, singly, or a pair at a time. They alighted in an inverted position, with wings flat against the bark. If at a distance from the froth, they approached by runs or short spurts, with a single flap of the wings at beginning or end. Occasionally a histerid beetle would push beneath the wings, causing a momentary flapping but not distracting the insect from its feeding. Once a *Colobura* alighted *on* the flattened wings, sending the insect into flight, but the Blue-spot returned and in turn drove away its annoyer.

The Red-banded Hindwing, *Biblis hyperia* (Cramer), is common about Simla but a rare visitor to the tangerine trees. Twice it was seen feeding on the froth, like *Peridromia* alighting upside down, with flattened wings.

On April 3, an individual of the Six Orange-spot, *Catonephila numilia* Cramer, came to the bait. This was a new record for Simla and Arima Valley. Two others were seen later.

The Zebra Clicker, *Colobura dirce dirce* (Linnaeus), was the only member of the tangerine group to be found commonly in the general vicinity at all times. During the present period of watching, from two to eight individuals were present at the bubbling areas. They had a regular flight routine, a quick, whirling dash, followed by a swoop to the bark of the nearest tree, alighting inverted, with closed wings. They often progressed by a series of short, quick runs over the branches. Zebra No. 1 had lost a full fourth of wing area, yet flew well.

It was present on the first day, March 6, and on the last, May 18, the same individual was seen feeding while perched on the wing cases of a large, brown, eyed-elater. This butterfly was among the most persistent in pushing aside the beetles and flies which interfered with its feeding. It not only pushed but slapped with sideways flicks of its tongue, to obtain for itself free access to the froth.

A medium-sized leaf-brown butterfly haunted the tangerines for a week before I could capture and identify it. It proved to be *Anaea morvus morvus* (Fabricius) deserving the vernacular name of the Tailed Pygmy Morpho. On alighting its wings snapped together and it became as much of a stemmed, dead leaf as any Kallima. Above, it was conspicuous, the proximal half of the wings iridescent morpho blue, the remainder black with two anterior, small, blue spots. This was the second specimen to be taken at Simla.

At first glance I thought that the family Ithomiidae must be included in the tangerine association, but a butterfly observed on March 14 proved to be another nymphalid—*Protogonius ochraceus* Butler, tailed and of typical ithomiid pattern. Several were observed, one of which crept close alongside a preponid and fed while brushing wings with the larger species.

Historis odium orion (Fabricius) was recorded six times, comprising at least four different individuals. In brief glimpses of the orange and black of upper wings in flight it resembled a brassolid; when alighted it closely approximated *Prepona.*

No day passed without one, two or all three species of preponids being present, feeding upon the frothy matter. These were *Prepona demophon* (Clerk), *Prepona antimache* (Hübner), and *Prepona meander* (Cramer). Owing to their swift flight among the lights and shadows it was impossible to differentiate the species on the wing, but after alighting, the under wing pattern was diagnostic. Especially was this true of *meander,* with the sharply demarcated halves of light and dark brown. All were seen to defend their position against encroachment by other butterflies or by beetles and flies. The tongues of preponids are pale red and seem stout enough to push and buffet aside any interfering insect. These usually wary butterflies were all exceedingly tolerant of approach, almost permitting one to touch them before taking flight.

Two individuals of the Trinidad Morpho, *Morpho peleides insularis* Fruhstorfer, were members of the tangerine association, coming to drink at odd times, and by sheer size and weight taking possession of some of the food areas.

Five Coconut Brassolids, *Brassolis sophorae sophorae* (Linnaeus), were visitors to the tangerines. Individual count was made possibly by the various degrees and positions of wing damage. Two of these insects made return visits throughout five weeks.

Two out of the three species of Trinidad Owl Butterflies or Caligos came to drink at the tangerine supply. These were *Caligo eurilochus minor* Kaye and *Caligo illioneus saltus* Kaye. These were easily identifiable on their alighting, because of their relative size, *minor* being appreciably the smaller. The ocellus in *saltus* is almost twice the size of the other. These great butterflies seldom came into contact with the others because of their crepuscular habits, arriving in early morning and in late evening.

B. Orders Other than Lepidoptera

Less attention was paid to orders other than Lepidoptera. They were few in number, both of species and individuals. All were drinking at the lesions.

1. Coleoptera

Large Green Elater, *Chalcolopidius virens* (Fabricius). Three were seen at feeding troughs on separate occasions. Did not seem to mind being picked up and replaced.

Large Brown Eyed-elater, *Pyrophorus pellucens* Eschscholtz. Two seen and captured.

Large Black Cetonia. One was captured on May 9 and another seen the following day.

Black Histerid Beetle. Feeding almost every day, usually in pairs.

Small Red-headed Histerid. Several every day.

2. Diptera

Stilt Fly, family Micropezidae, genus *Odontoloxozus,* species probably new. These were present in small numbers every day, two to six at each drinking station. They were very active, moving forward, backward and sideways so smoothly that they seemed to flow over the bark. In spite of being constantly flicked aside by the tongues of the butterflies, they persisted and occasionally took their stand *beneath* the bodies of the larger insects.

Hairy, Blue-bodied Fly, Tachinidae. Several seen.

Drosophila. A group of a dozen congregated at one source of nutriment during a week's time. Not seen at the other lesions.

3. Hymenoptera

Trigonid. Two seen, one taken. A large nest of these bees was established a few yards away, yet none, other than these two, was seen at the tangerines.

IV. Summary

Two unrelated plants, under very different conditions, have been found to be characterized by their pronounced selective power of attraction for a few definite groups of Lepidoptera.

The first is fedegoso or wild heliotrope, *Heliotropium indicum* Linnaeus, which exercises a powerful attraction for two families of butterflies, Danaidae and Ithomiidae, and two families of moths, Euchromidae and Arctiidae. These four families happen to be the most specialized in their respective groups. The attraction or lure becomes effective only on the death of the plant and after the consequent shrivelling and desiccation of the foliage. It persists for ten days or two weeks.

The second plant is the tangerine orange tree, *Citrus nobilis* Andre, afflicted with what is probably a virus disease. The lure is the fermented matter exuded from small, bark lesions. In this case the attraction extends to three families of Lepidoptera, Nymphalidae, Morphidae and Brassolidae.

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EXPLANATION OF THE PLATES

Plate I

Fig. 1. Fedegoso (*Heliotropium indicum* Linnaeus) growing wild.

Fig. 2. Terminal inflorescence of fedegoso.

Plate II

Fig. 3. Fedegoso suspended from a branch, with more than 30 butterflies feeding on the seed panicles.

Fig. 4. Ithomiid butterflies attracted to fedegoso.

Plate III

Fig. 5. Ithomiid butterfly, *Ithomia drymo pellucida* Weymer, male, with proboscis starting to uncoil, alighting on fedegoso.

Fig. 6. Day-flying euchromid moths feeding on seed panicle of fedegoso. Above: A wasp mimic, *Pseudosphex melanogen* Dyar, male. Below: *Dinia mena* Hübner, male.

Plate IV

Fig. 7. *Prepona antimache* (Hübner) feeding at tangerine lesion with histerid beetles.

Fig. 8. *Prepona meander* (Cramer) feeding at tangerine lesion with Stilt Flies, Micropezidae.

FIG. 1

FIG. 2

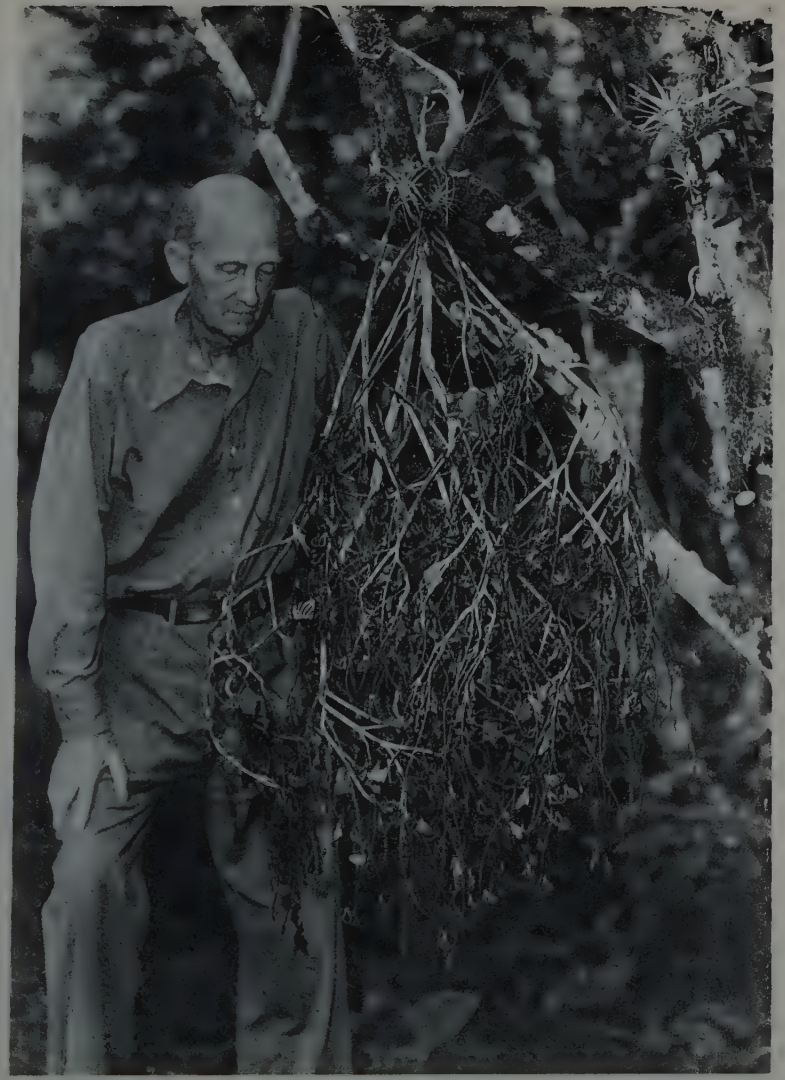

FIG. 3

FIG. 4

TWO LITTLE-KNOWN SELECTIVE INSECT ATTRACTANTS

FIG. 6

FIG. 5

TWO LITTLE-KNOWN SELECTIVE INSECT ATTRACTANTS

FIG. 8

FIG. 7

TWO LITTLE-KNOWN SELECTIVE INSECT ATTRACTANTS

3

A Factor Analysis of the Performance of Dogs on Certain Learning Tests[1]

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(Text-figures 1 & 2)

FACTOR analysis is a statistical technique for simplifying and clarifying the description of individual differences by reducing the number of necessary variables or dimensions. Such an analysis begins with the intercorrelations among a set of variables, such as the scores obtained by a group of individuals on a series of tests. The object of the analysis is to find the smallest number of factors or dimensions which can account for the obtained correlations among the test scores. Individual differences may then be described in terms of this relatively small number of factors, rather than in terms of all the original tests. For subsequent testing purposes, an effort is generally made to choose or develop single tests which provide the best measure of each of the factors identified in the analysis. Detailed discussions of the techniques of factor analysis and of its mathematical foundations have been given by Thurstone (1947), Holzinger & Harman (1941), Thomson (1951) and Cattell (1952). A very lucid elementary introduction to factorial techniques can be found in the recently published book by Fruchter (1954).

Although originally developed in connection with the study of human abilities, factor analysis has wide applicability. It has been employed in such diverse areas as the investigation of bodily physique and constitutional types, the classification of psychoses and neuroses, the identification of emotional and motivational traits, the study of interrelationships among allergy reactions, the exploration of aesthetic and humor preferences, the delineation of the cultural patterns of different nations and the analysis of the voting records of legislators and Supreme Court judges. For general surveys of the applications of factorial methods and for critical evaluations of results, reference may be made to Thurstone (1948), Fruchter (1954, Ch. 10), Anastasi (1948) and Anatasi & Foley (1949, Ch. 15). The special implications of factor analysis for test development are considered in Anastasi (1954, Ch. 14).

Factorial analyses of infrahuman behavior have been relatively few. A major reason for the infrequent use of this technique in animal studies stems from the difficulty of meeting certain important methodological requirements. Among such requirements, special mention should be made of the need for high test reliability, a sufficient number of variables to permit adequate determination and definition of each factor, and a large enough group of subjects so that chance errors of sampling will not loom too large in the correlation coefficients. Owing to their failure to meet one or more of these conditions, even the best available factorial investigations of infrahuman behavior must be regarded as preliminary and exploratory. And it should be added that such a characterization must also be applied to the study which will be reported in the present paper.

The pertinent animal studies published prior to 1950 have been summarized by Royce (1950a). About a dozen investigations conducted before 1935 reported correlations between two or more measures of learning. All were concerned with rats, with the exception of one study in which chicks were employed (Dunlap, 1933). The correlations were uniformly very low, except those between closely similar tasks, such as different mazes. There was no evidence of a general learning factor

[1] The raw data for this study were obtained at the Hamilton Station of the Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine, while the statistical analysis was conducted at Fordham University, New York City. The cost of IBM computations was covered partly by the Roscoe B. Jackson Memorial Laboratory and partly by the New York Zoological Society. Assistance in making many of the arrangements necessary to conduct this research was rendered by Dr. John V. Quaranta, formerly Research Associate in Animal Behavior, New York Zoological Society.

(Dunlap, 1933; McCulloch, 1935). To be sure, no common learning factor has been found in the case of human subjects either, the abilities or group factors identified in the human studies being organized along different lines. Thus a person who excels in spatial learning, for example, may be quite deficient in verbal or numerical learning. The early animal studies, however, showed little evidence of any sort of group factors beyond a few of very narrow scope. A high degree of specificity seemed to characterize the behavior measured in these studies.

The first systematic investigation of animal behavior by means of current procedures of factor analysis is to be found in a study of rat behavior by R. L. Thorndike (1935). A total of 32 scores was obtained from seven experimental set-ups, including mazes, problem boxes, conditioned response apparatus, activity wheel and an obstruction box for measuring the relative strength of different drives. The subjects were 64 albino rats. Factorial analyses indicated the presence of three factors, which were described as docility, transfer and a conditioned response factor.

Van Steenberg (1939) subsequently re-analyzed Thorndike's data and rotated the centroid axes for simple structure in accordance with the procedures developed by Thurstone. Such a rotation is now common practice in factorial studies, its object being to obtain a more clear-cut and easily interpretable configuration of factors. Van Steenberg's analysis yielded ten factors, five of which could, according to the author, be interpreted with some confidence. These factors were identified as follows: ability to profit from visual cues (common to elevated mazes), adaptability to new situations, speed of movement, ability to learn a right-left alternation and visual insight or perception of the total stimulus pattern. Of the remaining five factors, three were very narrow factors specific to one kind of apparatus; one admittedly defied psychological interpretation; and one was regarded as a residual factor. It should be added that the descriptions of the first five factors themselves fall somewhat short of desirable clarity. Nor does the extraction of ten factors from intercorrelations obtained on only 64 rats appear quite warranted.

In a later study, Vaughn (1937) applied centroid analysis and rotation of axes to the intercorrelations among a set of 34 measures obtained from 75 rats. An even wider variety of behavior was covered than had been the case in Thorndike's study, although most of the tests were again concerned primarily with learning. The apparatus included a wildness tunnel, an activity cage, a straightaway, a perseverance box, several types of mazes, a problem box and a test designed to measure reasoning. Eight factors were isolated, four of which were tentatively identified as follows: speed, wildness-timidity, associative or insight learning, and transfer.

Other ways in which factor analysis may be applied to the investigation of animal behavior are illustrated by the work of Wherry (1939, 1940, 1941) and Searle (1949). In Wherry's analyses, intercorrelations were found, not among the scores obtained by each animal, but among the numbers of errors made by the entire group in different segments of the learning situation. Thus each blind alley in a given maze was considered as an "individual," and the total number of entrances made during a given trial or stage of learning was taken as the "score" for that learning period. Intercorrelations of "scores" obtained in different periods were found and submitted to a centroid analysis, with subsequent rotation of axes. By this procedure, Wherry sought to investigate changes in the factorial composition of behavior at different stages of learning. When applied to published data from mazes and other types of learning situations, this procedure yielded remarkably consistent results. Factors described as forward-going, food-pointing and goal-gradient predominated in the initial, middle and final stages of learning, respectively.

Searle (1947, 1949) applied obverse[2] factor analysis to rat learning data. In this method, correlations are found between individuals rather than between tests or other variables. The procedure can be visualized if we think of the columns and rows of a table of scores as having been interchanged prior to the computation of intercorrelations. Each correlation thus obtained indicates the degree of similarity of the score patterns or profiles of two individuals. When such correlations are submitted to a factor analysis, the resulting factors represent clusters or "types" of individuals characterized by similar score profiles.

Factorial techniques have likewise been applied to the analysis of emotional and motivational data obtained in animal studies. But the results in this area are even more tentative than those in the field of learning. Geier, Levin & Tolman (1941) factor-analyzed 29 measures of the behavior of 57 rats in two experimental set-ups. Both learning and emotionality indices

[2] Also known as "Q-technique" and sometimes incorrectly described as "inverted factor analysis." In the terminology of matrix algebra, such an analysis involves, not the inverse, but the transpose of the original score matrix.

were represented in this study. An investigation concerned only with the factorial composition of emotionality indices was conducted on 40 rats by Billingslea (1942). Using a procedure similar to that of Wherry, described above, Rethlingshafer (1941) compared the factorial composition of different stages of learning under conditions of varying motivational strength. Previously published data on rats were utilized for this purpose.

More recently, Royce (1950b, 1951) applied the centroid method of factor analysis to the intercorrelations among 32 physiological, psychological and social measures of emotionality obtained from 53 dogs. Rotation of the centroid axes yielded an oblique simple structure. Of the ten factors thus identified, six were tentatively interpreted as follows: psychophysiological timidity, behavioral timidity, heart reactivity to social stimulation, aggressiveness, activity level and audiogenic reactivity. Many of the animals utilized in the Royce investigation have been included in the sample employed in the present study.

Procedure

The present study represents an exploratory factorial analysis of the performance of dogs in a variety of learning situations. The data were gathered by members of the research staff of the Division of Behavior Studies, Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine, as part of a long-range project on genetics and social behavior in dogs. A brief account of the over-all research plan can be found in a report by Scott & Fuller (1951). For descriptions of the physical environment and of the procedures followed in the care and rearing of the dogs, the reader is referred to the *Manual of Dog Testing Techniques*, edited by Scott & Fuller (1950, pp. 4-9).

Subjects. — Seventy-three dogs of pedigreed stock were included in the present sample. All had been reared under uniform laboratory conditions and had been put through a standardized system of handling, training and testing. Detailed genetic records on each animal are available at the Jackson Laboratory.

Table 1 shows the breed and sex distribution of the subjects. It will be noted that the group comprised 16 Basenjis, 4 Beagles, 18 Cocker Spaniels, 5 Shetland Sheep Dogs, 7 Wire-haired Fox Terriers and 23 Basenji-Cocker Spaniel crosses. There was a total of 34 males and 39 females. The animals employed represent all those for whom complete data were available on the variables under consideration.

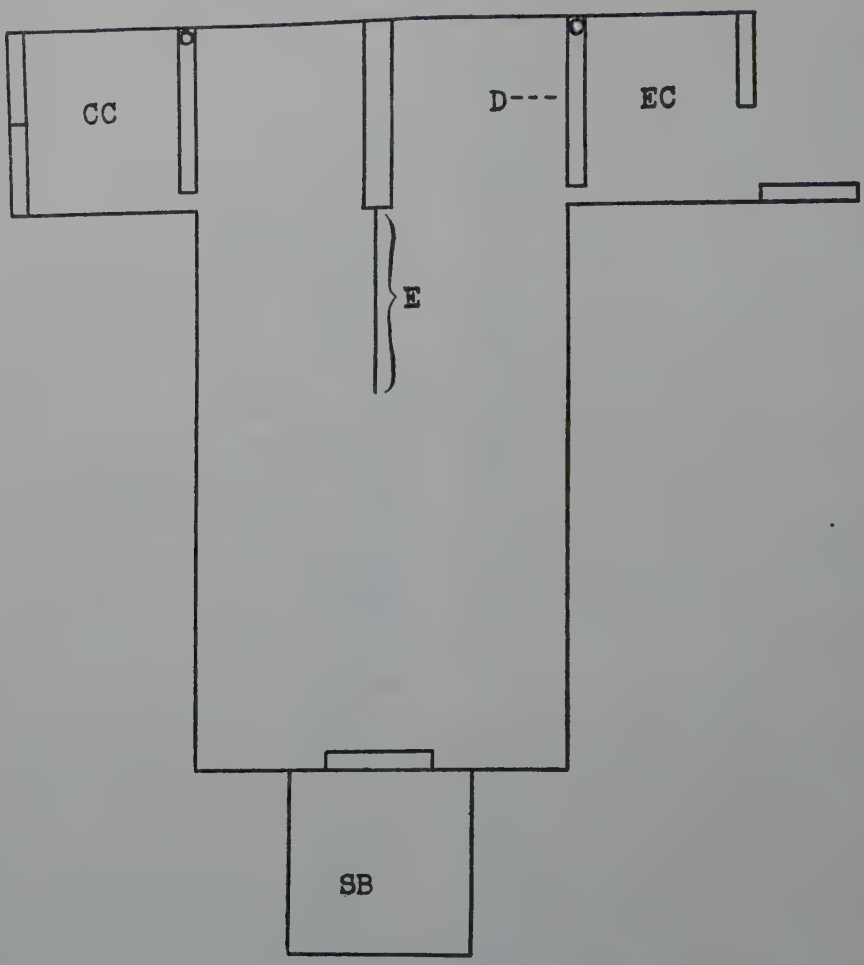
Tests.—The present analysis is based on the scores obtained in the 17 variables described below.[3] More detailed descriptions of the tests from which these scores were derived are provided in the previously cited *Manual of Dog Testing Techniques* (Scott & Fuller, 1950). In order to facilitate cross-references, the test names given in the manual have been employed in the present report, even when an objective examination of the test might suggest the desirability of a somewhat different name.

Table 1. Breed and Sex Distribution of Subjects

Breed	Male	Female	Total
Basenji	8	8	16
Beagle	3	1	4
Cocker Spaniel	8	10	18
Shetland Sheep Dog	3	2	5
Wire-haired Fox Terrier	2	5	7
Basenji-Cocker Spaniel Cross	10	13	23
Total	34	39	73

1. *Habit Formation: Time.*—The dog is placed in a small release cage, while a food box is placed a few feet away. The release box is remotely operated by the unseen observer. Two trials a day are given over a five-day period, the food box being placed in one position for the first two days and in another position during the last three. The score is the total time required to reach the food box in ten trials.
2. *Manipulation: Time.*—The same apparatus is used as in variable 1, except that the dog cannot reach the food without first biting, nosing, or pawing the food dish out of the food box. Two trials a day are given for two days. The score is the total time required to obtain the food in four trials.
3. *Manipulation (String-pulling): Time.* — The same apparatus is again used as in variables 1 and 2, except that the food dish is placed well back in the food box and must be pulled out by a string which is attached to the rim of the dish. The score is the total time required to obtain the food in two trials.
4. *Maze (Second Barrier Test): Errors.*—The apparatus for this test consists of a six-unit T-maze with a food dish at the exit. Since the barriers are made of poultry netting, the solution to each part of the maze is visible, but not that of the whole. Following a two-day orientation period, each dog is put through one trial

[3] Other variables were considered and discarded because of lack of experimental independence of scores. The measures omitted for this reason include a cover-lifting test and three scores obtained in a discrimination and delayed-response apparatus. In all these tests, subjects who had failed an earlier related test were not subsequently tested, but were automatically recorded as failures in the new test.



TEXT-FIG. 1. Discrimination apparatus. SB—Starting Box. EC—Escape Corridor. CC—Closed Corridor. D—Door banged as cue. E—Partition which is swung either right or left in variable 5 (Motivation) so as to completely obstruct one corridor.

a day for ten days. An error is recorded whenever an animal stops or reverses direction. The score is the total number of errors in the last nine of the ten trials.

5. *Motivation* (*Discrimination Apparatus*): *Time.*—This test was designed to measure the motivational strength of each animal, as indicated by his running time in escaping from an enclosed area and in reaching food. The discrimination apparatus (cf. Text-fig. 1) is utilized as the enclosed area. This apparatus has a starting box and two escape corridors. In the motivation test, one escape corridor is completely closed off so that the dog has only one possible route. To provide a visual and auditory cue, the experimenter bangs the inner door of the correct escape corridor four or five times, just as he releases the dog from the starting box. After escaping from the apparatus, the dog is allowed to run freely in the room and is fed by the experimenter near the starting box. The order of presentation of escape corridors (RLRRLRLLRL) is designed to avoid the formation of position habits or of a simple alternation habit. The score is the median time required to escape in ten trials.

6. *Cue Response* (*Discrimination Apparatus*): *Trials.*—Cue response training is started on the day following completion of the motivation test. The apparatus is arranged so that the center partition extends directly forward, as shown in Text-fig. 1, rather than being swung right or left as in the motivation test. The dog must therefore choose one side or the other before he can see which outer door is open. Entrance into the wrong corridor counts as an error. The cue is given as in the motivation test by swinging the inner corridor door four or five times and releasing the dog from the starting box immediately thereafter. The outer door of the uncued corridor is closed so that the dog can never escape from the wrong corridor. A

uniform random sequence of right and left escape corridors is again used. The score is the number of trials required to reach either of two pre-established criteria of learning in which the proportion or sequence of correct choices exceeds chance at the .01 level of significance.

7. *Cue Response* (*Discrimination Apparatus*): *Time.*—This variable is the same as variable 6, except in the scoring. In the present variable, the score is the median time required for the last 19 correct trials of cue response training.

8. *Leash Control* (*In*): *Trials.*—The dog is removed from the outside pen before the leash is put on him. He is then led on leash over a short course of outdoor pathways and brought into the building, where he is fed a small amount of fish. The training is continued for ten days. The dog's performance is rated by assigning differential weights to errors of varying degrees of seriousness, including balking, pulling, dragging, fighting the leash, "sunfishing," crossing in front of or jumping at the trainer and various kinds of vocalizations such as whining, yelping or howling. The score is the number of trials required to reach a performance rating of 2 or less.

9. *Leash Control* (*Stairs*): *Trials.*—During the last eight days of leash control training, the dog is also taken through the building and up the stairs to one of the lofts, where he is fed. The course includes a flight of stairs interrupted by a landing. A rating scale similar to that of variable 8 is used to score the subject's performance while climbing up and down the stairs. The score is the number of trials required to reach a performance rating of 2 or less on this portion of the course.

10. *Leash Control* (*In*): *Initial Errors.*—This variable is the same as variable 8, except in the scoring. The score is the error rating obtained on the first day of training in leash control.

11. *Leash Control* (*Stairs*): *Initial Errors.*—This variable is the same as variable 9, except in the scoring. The score is the error rating obtained on the first day of training in stair climbing.

12. *Motor Skills*: *Time.*—This test was designed to measure the dog's general physical skill, especially in relation to climbing, jumping and balancing. Two boxes, each one foot high, are stacked and a two-by-five-foot ramp leads to the top box where a food dish is placed. The dog is released about six feet from the apparatus and is timed from his release to the moment when he reaches the food. The score is the total time on three trials.

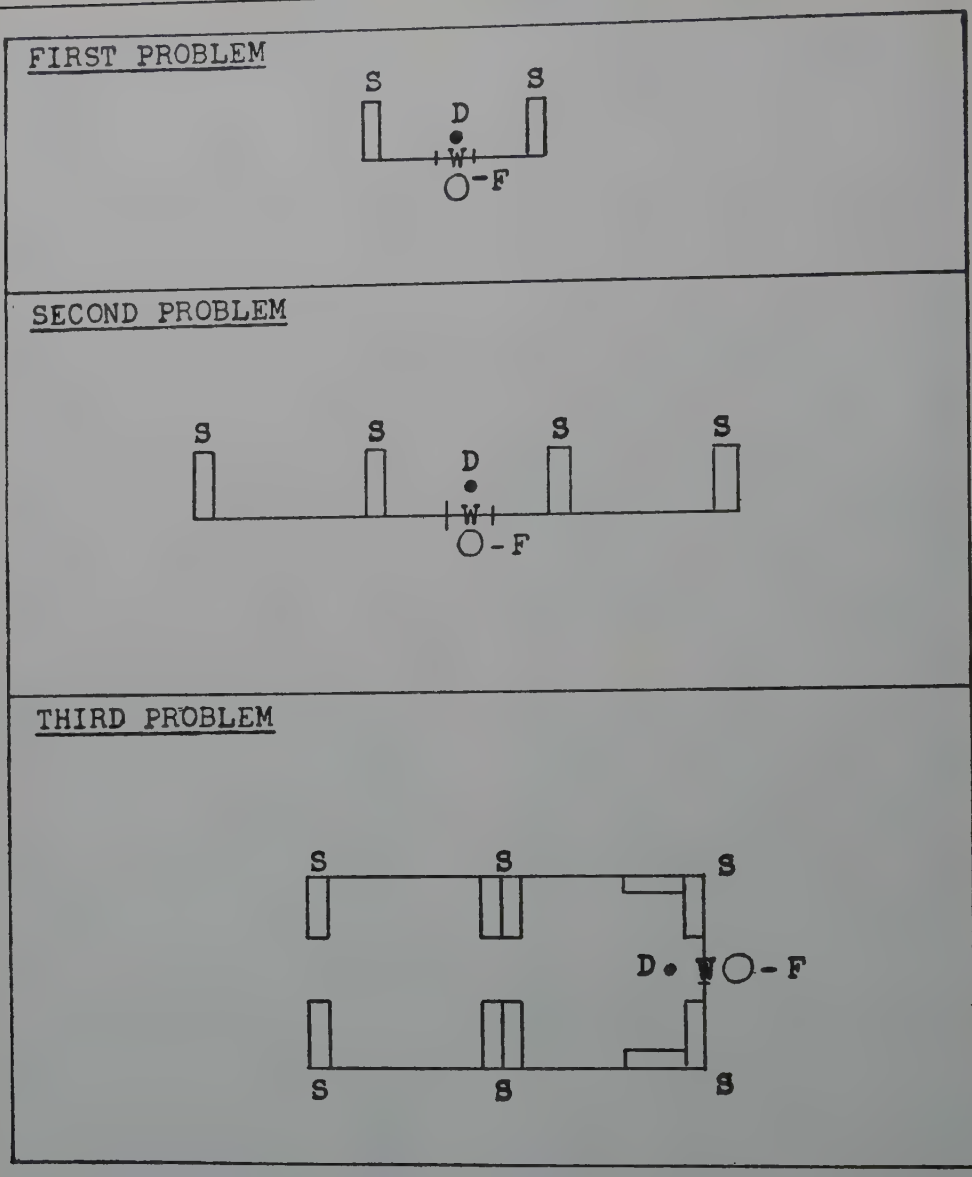
13. *First Barrier Test* (*First Problem*): *Errors.*—This test is of the "Umweg" type and was designed to test performance in a situation which is totally new to the dog. It may also test generalization or transfer of training from a simple situation to a more complex one of the same type, as represented by variables 14 and 15. The test is conducted within a large rectangular area, two days being initially employed to accustom the animal to this area. The barriers consist of five wood-and-wire fences, six by three feet, with supports on one side. Each is covered with opaque brown paper, except for a one-foot-wide window in the center of the barrier behind which the dog is placed. The barriers are always set up so that the supports are on the side on which the dog is placed. In the present variable, only one barrier is used. The dog is first allowed to smell the food and is then placed on the opposite side of the barrier, where he can see the food through the window (cf. Text-fig. 2—First Problem). The dog can solve the problem by taking either of the two possible paths to the food. An error is recorded whenever the animal stops or reverses direction. The score is the total number of errors on three trials.

14. *First Barrier Test* (*Second Problem*): *Errors.*—This variable is the same as variable 13, except that three barriers are placed end to end so as to form a longer straight-line obstruction (cf. Text-fig. 2—Second Problem). The score is the total number of errors on three trials.

15. *First Barrier Test* (*Third Problem*): *Errors.*—The procedure and apparatus are again the same as those described under variable 13, except that five barriers are set up in a U shape (cf. Text-fig. 2—Third Problem). The score is the total number of errors on three trials.

16. *Obedience* (*Adjusted Stay Score*): *Time.*—A choke collar with a short lead is placed on the dog and he is led to a box which is 20 inches high and 20 × 16 inches on the top. The dog is lifted to the top of the box and given the command, "Stay." The lead is held so that the dog is choked if he leaps from the box. When the animal learns to remain on the box for 30 seconds, training for responding to "Down" is begun. When he stays up for 30 seconds and jumps promptly at "Down," training without a collar is started. The experimenter stands within 6 inches of the box but does not touch the dog or restrain him from jumping. If the dog remains on the box for 30 seconds and jumps promptly at "Down," the distance of the experimenter is increased on the next trial. The control distances are: 6″, 18″, 36″, 72″, 144″, and out of sight behind a screen placed 14 feet from the box (BHS). A total of three days is devoted to the above training. On the fourth or test day, the dogs are tested in the following sequence of control distances: 6″, 18″, 72″, 144″, BHS, 6″, 18″, 72″, 144″, BHS. The score employed in this variable is the "adjusted stay score," i.e., the total time during which the dog remains on the box in the ten 30-second test trials (max. = 300 sec.), minus a 10-second penalty for each failure to jump within 10 seconds of the command "Down."

17. *Obedience*: *Jumps during Training.*—This variable is similar to variable 16, the score being



TEXT-FIG. 2. First barrier test. S—Supports for barriers. F—Food dish. D—Dog. W—Window in barrier.

the number of spontaneous jumps with collar on, or at the minimum distance of 6 inches without the collar, during the *training period.* Each such jump constitutes an error.

RESULTS

Conversion of Scores.—Prior to the computation of intercorrelations, the scores on all variables except two (variables 3 and 6) were converted to single-digit, normalized standard scores.[4] The converted scale ranges from 0 to 9, with a mean of 4.5 and a standard deviation of 2. It will be recalled that the raw scores on all variables except 16, the adjusted stay scores on the obedience tests, were expressed so that the higher the score the poorer the performance. In the converted scores, however, 9 represents the best performance and 0 the poorest in all variables.

Some of the converted distributions retained a certain amount of skewness or other irregularities. Such variations result from the occurrence of an excessive number of identical scores either at the upper or lower end, or at some

[4] A list of raw scores, as well as details of the score conversion and other computational procedures, can be found in Schmitt (1954).

other part of the range. Since all such identical scores were assigned the same converted score, the frequency of a given converted score sometimes exceeded that required by the normal curve transformation. Nevertheless, the converted distributions of 15 variables were deemed to be sufficiently close to a normal curve for use in the computation of Pearson correlation coefficients. In the case of the remaining two variables, however, the marked skewness resulting from the large number of failures led to the decision to dichotomize the variables. This was done for variable 3, string pulling, and variable 6, trials to learn cue response.

Intercorrelations.—The intercorrelations among the 17 variables were computed by IBM procedures at the Test Division of The Psychological Corporation, New York City. All are Pearson correlations, except that between variables 3 and 6, which is tetrachoric, and those between variables 3 or 6 and the remaining variables, which are biserial. The complete set of 136 correlations is reproduced in Table 2. The correlations range from +.71 to −.43, including 82 positive and 54 negative coefficients. For a sample of 73 cases, the minimum correlations significant at the .05 and .01 levels are ±.232 and ±.302, respectively. Reference to Table 2 shows that 44 coefficients reach or exceed the .05 level of significance; and of these, 27 reach or exceed the .01 level. By chance, between 6 and 7 of the 136 correlations would be expected to reach the .05 level, and only 1 or 2 of these should reach the .01 level.

Factor Analysis.—The intercorrelations were analyzed by Thurstone's complete centroid method (Thurstone, 1947, Ch. 8). The criterion employed for determining how many factors to extract was that developed by McNemar (1942). According to this criterion, the *sth* factor is significant if the estimated SD of the partial correlations remaining after the extraction of s factors exceeds the standard error of a zero correlation. The SD of the partial correlations (σ_s) is estimated by the following formula: $\sigma_s = \frac{{}_s\sigma_\rho}{1 - M_{h_s^2}}$, in which ${}_s\sigma_\rho$ is the SD of the *sth* factor residuals and $M_{h_s^2}$ is the mean communality of s factors. With 73 cases, the standard error of a zero correlation is .1179. This value is slightly less than that of σ_{IV} (.1257), but exceeds that of σ_V (.1168). Factorization was therefore discontinued after the extraction of the 5th factor.

In Table 3 will be found the centroid factor matrix, showing the weight of each of the five factors in each of the 17 variables, as well as the communality, or proportion of common factor variance, in each variable. The centroid axes were next rotated graphically in such a way as to maximize the number of zero factor loadings (simple structure), while retaining the orthogonal relationship among the axes. The rotated factor matrix is reproduced in Table 4.

It will be noted that the mean communality is .46. Factor II contributes the largest proportion of common variance, .12. Factors I and IV each contribute .10; and Factor V accounts for .09. The smallest contribution, .05, is made by Factor III. The uniqueness of the variables, including unknown proportions of specificity and error variance, accounts for as much as 64 per cent of the total variance of the battery.

Interpretation of Factors.—In order to arrive at a provisional psychological interpretation of each of the five rotated factors, all variables having loadings of ±.40 or higher on that factor were examined. Such a factor loading accounts for 16% or more of the variance of the particular variable.

Reference to Table 4 shows that the variables which meet the above criterion with regard to *Factor I* are the following:

13. First Barrier Test (First Problem): Errors	.47
11. Leash Control (Stairs): Initial Errors	.47
9. Leash Control (Stairs): Trials	.46
16. Obedience (Adjusted Stay Score): Time	−.44
17. Obedience: Jumps during Training	−.53

The type of behavior involved in all these tests suggests that Factor I may be related to *activity* and *impulsiveness.* In the first barrier problem, the more active or impulsive animal is less likely to hesitate or reverse direction in going to the food dish. It might be added that the second and third barrier problems (variables 14 and 15) also show appreciable positive loadings on Factor I, but of decreasing magnitude (.39 and .31). These two problems would also favor the more impulsive animal, since hesitations and reversals again constitute the only errors. Lower loadings would be expected, however, than on the first problem, which represents the animal's initial contact with a relatively strange situation. Moreover, because of their greater complexity, the second and third problems may depend more heavily upon cognitive factors than upon mere impulsiveness or general activity level.

In the two measures of stair climbing (variables 9 and 11), the more active animal is less likely to manifest such behavior as balking and dragging, both of which are scored as errors.

TABLE 2. INTERCORRELATIONS AMONG THE SEVENTEEN VARIABLES
(N = 73)

Variable	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1																
2	.3303															
3	.3902*	.4696*														
4	.0460	−.1692	−.1898*													
5	−.0284	.1025	−.1539*	−.1381												
6	.1949*	.1949*	.0700†	−.4338*	.5173*											
7	−.0392	.0757	−.0624*	−.0294	.7070	.4902*										
8	−.3525	−.1610	−.2692*	−.0684	.4492	.0302*	.2724									
9	.1433	.1327	.0102*	.0410	.0892	.2716*	.1344	.0242								
10	−.1273	−.0261	−.0892*	−.1190	.4002	.1678*	.2641	.3859	−.0241							
11	.0075	−.0510	.1112*	−.1822	.2680	.2943*	.2383	.3073	.2443	.3079						
12	.3101	.2026	.0792*	−.0087	.0387	−.0035*	.0345	−.0778	−.0462	.0651	.1001					
13	.2990	.0886	.3705*	−.2212	−.0578	.1461*	.0208	−.3031	.2726	.0325	.1793	.2023				
14	.3860	.0966	.1477*	.0437	−.3880	.0394*	−.2719	−.2195	.2254	−.1025	.1202	.0546	.4058			
15	.2239	.0406	.1487*	−.1704	−.2200	.1713*	−.3075	−.3537	.0679	−.1757	.1395	.1595	.2811	.4291		
16	−.0393	.0075	−.0151*	−.0094	.1618	.1390*	.1668	.1981	−.2648	.1630	−.1155	−.1185	−.1508	−.2296	−.2900	
17	−.1528	−.0829	−.0631*	.2475	.1975	−.1400*	.1859	.2995	−.2785	.3034	.0108	−.1704	−.3424	−.3948	−.3641	.4866

All correlations are Pearson Product-Moment Coefficients unless otherwise indicated.
* Biserial correlation.
† Tetrachoric correlation.

TABLE 3. CENTROID FACTOR MATRIX

Variable	Factor					h^2
	I	II	III	IV	V	
1. Habit Formation: Time	.5116	.2032	−.3358	.3516	.1556	.5636
2. Manipulation: Time	.3383	.3634	−.3985	−.1795	−.1319	.4549
3. Manipulation (String pulling): Time	.4722	.2973	−.4206	−.2253	.0706	.5440
4. Maze (Second Barrier Test): Errors	−.2371	−.3162	−.1768	.1848	.3375	.3355
5. Motivation (Discrim. Appar.): Time	−.3519	.7085	.2551	.1168	−.2046	.7464
6. Cue Response (Discrim. Appar.): Trials	.1709	.6063	.3021	.1391	−.4237	.6869
7. Cue Response (Discrim. Appar.): Time	−.2978	.6478	.1535	.1362	−.1217	.5653
8. Leash Control (In): Trials	−.5104	.3040	.2917	−.2519	.1920	.5383
9. Leash Control (Stairs): Trials	.2853	.1631	.3100	−.1146	.1405	.2370
10. Leash Control (In): Initial Errors	−.2687	.4404	.1671	.0547	.2501	.3596
11. Leash Control (Stairs): Initial Errors	.0940	.4113	.3916	−.0576	.3299	.4435
12. Motor Skills: Time	.2439	.1531	−.1104	.1340	.0764	.1189
13. First Barrier Test (First Problem): Errors	.5578	.2211	.1079	.1337	.2011	.4300
14. First Barrier Test (Second Problem): Errors	.5917	−.1248	.1292	.1608	.2805	.4869
15. First Barrier Test (Third Problem): Errors	.6032	−.1463	.1804	.2085	−.0697	.4661
16. Obedience (Adjusted Stay Score): Time	−.4147	.2231	−.2711	.1944	−.0679	.3376
17. Obedience: Jumps during Training	−.6356	.1675	−.3209	.0961	.1735	.5744

On the other hand, the negative weights of the two obedience measures are understandable, since an active, impulsive dog is more likely to jump down and finds it more difficult to remain motionless for the required period. It may also be suggested, as a further elaboration or description of Factor I, that this factor indicates confidence in a strange situation and lack of timidity. It is noteworthy in this connection that all three variables which have high positive loadings on this factor are based on tests administered outside the animal's normal living environment.

Turning our attention to *Factor II*, we find loadings of .40 or more on the following variables:

8. Leash Control (In): Trials	.57
10. Leash Control (In): Initial Errors	.56
17. Obedience: Jumps during Training	.54
5. Motivation (Discrimination Apparatus): Time	.54
7. Cue Response (Discrimination Apparatus): Time	.53
11. Leash Control (Stairs): Initial Errors	.40
15. First Barrier Test (Third Problem): Errors	−.45

This factor appears to involve *docility* or *responsiveness to a human trainer.* Its two highest positive weights occur in those measures of leash control in which the animal is led over an outdoor course. A loading of .40 is likewise found in initial errors made when being led up the stairs on leash. Subsequent stair-climbing performance, however, shows no significant loading with this factor, probably because such performance soon becomes primarily a matter of motor skill or activity rather than responsiveness to the trainer. Similarly, the measure based upon performance during obedience training (variable 17) has a loading of .54 on this factor. A lower loading of .32, which may also be significant, is found in the performance measure obtained after completion of obedience training (variable 16).

The motivation and cue response (time) measures both involve speed of escaping from the discrimination apparatus. It will be noted that the pattern of weights on all five factors is closely similar for these two variables. With reference to the present factor, it should be recalled that in both tests the animal receives food from the experimenter, whom he must approach specially for this purpose, since the experimenter does not stand near the exit of the apparatus. Relation to the human trainer thus appears to play a more important role in these tests than in those in which the animal obtains food impersonally from a dish.

In this connection it is also interesting to observe the negative weight on the third barrier problem. The first two barrier problems likewise have negative weights on this factor, although the weight is negligible for the first problem. It

TABLE 4. ROTATED ORTHOGONAL FACTOR MATRIX

Variable	Factor					h^2
	I	II	III	IV	V	
1. Habit Formation: Time	.10	−.04	.00	.73	.00	.56
2. Manipulation: Time	.20	.00	.41	.37	−.32	.45
3. Manipulation (String pulling): Time	.31	.00	.46	.46	−.13	.54
4. Maze (Second Barrier Test): Errors	−.34	.09	.00	.03	.47	.35
5. Motivation (Discrim. Appar.): Time	.01	.54	−.27	−.13	−.60	.74
6. Cue Response (Discrim. Appar.): Trials	.26	.09	−.31	.08	−.71	.68
7. Cue Response (Discrim. Appar.): Time	−.02	.53	−.20	−.04	−.50	.57
8. Leash Control (In): Trials	.05	.57	−.02	−.46	−.04	.54
9. Leash Control (Stairs): Trials	.46	.03	−.12	.00	.01	.23
10. Leash Control (In): Initial Errors	.07	.56	−.15	−.06	−.08	.35
11. Leash Control (Stairs): Initial Errors	.47	.40	−.23	−.02	.00	.43
12. Motor Skills: Time	.11	.02	−.01	.32	−.03	.12
13. First Barrier Test (First Problem): Errors	.47	−.03	−.17	.42	.02	.43
14. First Barrier Test (Second Problem): Errors	.39	−.24	−.20	.37	.29	.47
15. First Barrier Test (Third Problem): Errors	.31	−.45	−.28	.30	.04	.47
16. Obedience (Adjusted Stay Score): Time	−.44	.32	.06	.04	−.19	.34
17. Obedience: Jumps during Training	−.53	.54	.16	−.06	.03	.60
$\frac{\Sigma a^2}{n}$	.10	.12	.05	.10	.09	.46

will be recalled that in all three barrier problems, the animal must walk away from the food in order to circumvent the barrier. In the second problem he must walk farther than in the first; and in the third, which presents a U-shaped barrier, he must turn completely around and walk in the direction opposite to that of the food. Moreover, in all these problems, the experimenter sits by the food dish and is visible through the window in the screen. An animal which is unduly dependent upon the human trainer might thus be handicapped in these problems—and particularly on the third—since the correct solution requires that he begin by walking away from the visible experimenter. The less docile and more "socially independent" animal, on the other hand, tends to respond to the physical elements of the situation, with little or no regard for the position of the experimenter.

The only variables which meet our criterion for the interpretation of *Factor III* are:

3. Manipulation (String pulling): Time .46
2. Manipulation: Time .41

The factor may thus be named *manipulation*, in the sense of pawing, nosing, biting or pulling with the teeth. The measures listed above are the only variables which require such activities. To be sure, this factor is underdetermined, insofar as it has weights of .40 or more in only two variables. At the same time, the proposed interpretation of this factor is supported by the consistent pattern of low negative weights in variables involving the discrimination apparatus, leash control and the barrier problems. In all these tasks, any biting, nosing or pawing behavior would delay the animal, distract him from the correct solution, or might in some cases be counted directly as an error, as when the animal bites or fights the leash.

On *Factor IV*, the following variables have loadings of .40 or more:

1. Habit Formation: Time .73
3. Manipulation (String pulling): Time .46
13. First Barrier Test (First Problem): Errors .42

8. Leash Control (In): Trials −.46

Since all tests with high positive loadings on this factor require the use of vision in locating objects or in perceiving the relationships among objects, the factor may be identified as *visual observation*. In the habit formation test, the location of the food dish is changed from trial to trial, so that the animal must be guided by visual cues in order to reach the incentive. In the string-pulling test, the discovery of the string and its proper utilization to secure the

food depend upon visual observation. It will be noted that the other manipulation test (variable 2) also has an appreciable positive weight of .37 on this factor. Similarly, all three problems of the first barrier test require the correct visual perception of the spatial relations between barrier and goal. The first of these problems has a weight of .43 on this factor. The second and third have weights of .37 and .30, respectively. Although the three successive problems are of increasing difficulty, it is possible that the benefit to be derived from visual observation is greatest in the initial problem, when the animal must first discover the Umweg type of solution to be followed.

It should also be noted that the motor skills test has a loading of .32 on this factor. Although this is not a high weight, it is the highest loading of this test with any factor, all other loadings being virtually negligible. In this test, too, the dog must correctly observe the relation of ramp to food dish. And he must inhibit any tendency to try to reach the food by jumping directly from the ground to the stacked boxes, rather than by climbing the ramp. In this respect the motor skills test might be said to require that the animal visually recognize an Umweg-type solution.

The negative weight of Factor IV in the single measure of leash control (variable 8) suggests the possibility that the more visually observant animal is more likely to be distracted and hence drag, pull, or make similar errors. Visual distractions of interest to the dog would probably occur more often in the outdoor course followed in variable 8 than in stair-climbing (variables 9 and 11). Similarly, such distractions would not be likely to operate on the first day of leash training (variable 10), since the animal's attention would then be more completely absorbed by the novelty of the leash itself.

The variables to be considered in the interpretation of *Factor V* include:

4. Maze (Second Barrier Test): Errors	.47
7. Cue Response (Discrimination Apparatus): Time	—.50
5. Motivation (Discrimination Apparatus): Time	—.60
6. Cue Response (Discrimination Apparatus): Trials	—.71

The animal which performs well on the maze is probably one who has good positional memory for the correct turns. Conversely, the tendency to take the same path on successive trials is a handicap on all three variables based on the discrimination apparatus, since the correct escape route is varied in random order from trial to trial. It would thus seem that Factor V represents *persistence of positional habits.*

Breed Differences.—It should be borne in mind that some of the factors which have been identified may correspond to characteristic differences among the breeds included in the present sample. Previously published studies on many of the same dogs employed in the current investigation provide evidence of significant physiological differences among these breeds (Fuller, 1951). Observations of the general behavior of the dogs have likewise suggested breed differences in such traits as timidity, attraction to human handlers and activity level (Scott & Charles, 1953). Analyses of breed differences have also been carried out on four of the tests included in the present study, viz., Maze (Scott & Charles, 1953), Motivation (Fuller, 1953), Cue Response (Fuller & Scott, 1954) and Leash Control (Fuller & Scott, 1954). In all of these variables, one or more significant differences between breeds were found.

The number of cases available for these analyses was small, especially in certain breeds. In the current study, some of these numbers were further reduced by the necessity of retaining only animals with complete records on all 17 variables. Nevertheless, it may be of interest to examine the results on breed differences in the present group. The relevant data are summarized in Tables 5 and 6, covering continuous and dichotomized variables, respectively. In Table 5, the results are reported in the form of median scaled scores for each breed. It will be recalled that the unit employed in these scaled scores is .5SD. Table 6 gives the median raw score, as well as the number of cases passing and the number failing each test.

Reference to Tables 5 and 6 suggests that the Basenjis tend to excel in tasks requiring independent action and visual observation of relations, such as habit formation, manipulation, string pulling, the three barrier problems and the maze. They are especially deficient in tasks which depend upon responsiveness to the human handler, such as leash control and obedience training. And they also do poorly on the discrimination apparatus tests. It is interesting to note that the inferiority of the Basenjis on some of these tests is so pronounced that there is no overlapping with the distributions of high-ranking breeds. This is true of Basenji-versus-Beagle in the motivation test (variable 5), and of Basenji-versus-Wire-haired Fox Terrier in leash control (variable 8). Thus in these two variables the best

TABLE 5. MEDIAN SCALED SCORES FOR EACH BREED: CONTINUOUS VARIABLES*

Variable	Bas.	Bea.	CS	SS	WHT	Bas. × CS
1. Habit Formation: Time	5	4	4	2	4	5
2. Manipulation: Time	6	4.5	4	5	5	4
4. Maze (Second Barrier Test): Errors	5	6	5	3	2	4
5. Motivation (Discrim. Appar.): Time	3	7	5	5	6	4
7. Cue Response (Discrim. Appar.): Time	3	7.5	5	4	5	4
8. Leash Control (In): Trials	2	6	5	6	5	4
9. Leash Control (Stairs): Trials	5	7	2.5	5	5	4
10. Leash Control (In): Initial Errors	4	5.5	6	2	6	4
11. Leash Control (Stairs): Initial Errors	4	6.5	4	4	6	6
12. Motor Skills: Time	4	4	5	3	3	6
13. First Barrier Test (First Problem): Errors	5	6.5	3	4	6	5
14. First Barrier Test (Second Problem): Errors	7	3.5	4	2	5	5
15. First Barrier Test (Third Problem): Errors	5.5	4.5	3	5	4	5
16. Obedience (Adjusted Stay Score): Time	3	4.5	5	6	5	4
17. Obedience: Jumps during Training	4	5	6.5	2	4	4
Number of Cases	16	4	18	5	7	23

* High scores signify better performance. All scores are scaled to an over-all M of 4.5 and σ of 2.

Basenji score falls at least .5SD below the poorest Beagle or Terrier score, respectively.

Since there were only 4 Beagles in the group, it is especially hazardous to make any statements about their performance. The exceptionally high achievement level of these dogs on several of the tests, however, is very striking. This is particularly evident in the three discrimination apparatus tests and in leash control. The Cocker Spaniels excel in obedience training and leash control, but not in stair climbing, which yields their poorest score. They are also poor in cue response (trials), which requires the establishment of an association between visuo-auditory cue and open exit; but they do relatively well on the other two discrimination apparatus tests. In string pulling, they exhibit the poorest performance in the group; and they are also below average in the manipulation test. Any conclusions about the Shetland Sheep

TABLE 6. ANALYSIS OF BREED DIFFERENCES IN DICHOTOMIZED VARIABLES

Variable	Bas.	Bea.	CS	SS	WHT	Bas. × CS
3. Manipulation (String Pulling)						
No. failing	2	1	9	2	3	4
No. passing	14	3	9	3	4	19
Median* time in seconds	124	276	477†	199	430	262
6. Cue Response (Discrim. Appar.): Trials						
No. failing	5	0	7	1	0	5
No. passing	11	4	11	4	7	18
Median* No. of Trials	113	27	116	30	48	84
Number of Cases	16	4	18	5	7	23

* The failures were included in the computations of these medians. The higher the raw scores, the poorer the performance.

† This median falls midway between a bona fide score and a failure, which was automatically recorded as 480.

Dogs must be very tentative because of the small number of cases. The outstanding finding regarding this breed seems to be its relatively poor performance on many variables.

The Wire-haired Fox Terriers achieve their best scores on tests which seem to call for confidence in strange situations. These are illustrated by the motivation test (which represents the animal's first contact with the discrimination apparatus), initial errors in both leash control and stair climbing, and the first barrier problem. They also do well in other tests involving the discrimination apparatus. On the other hand, they do particularly poorly on the maze, where it is reported that they become over-excited and make many errors (Scott & Charles, 1953). Little can be concluded regarding the Basenji-Cocker Spaniel crosses beyond the fact that they are close to the total group mean on most measures.

A sharper delineation of breed differences in behavior characteristics might be obtained through the application of obverse factor analysis or Q-technique. For this purpose, it would be desirable to have scores on a more extensive set of variables, or at least more part-scores resulting from further breakdowns of the present variables. Eventually it would be advisable to carry out factor analyses similar to that reported in the present study on each breed separately. This would, of course, require a much larger sampling of each breed than is now available.

Summary and Conclusions

The scores of 73 pedigreed dogs on 17 variables, most of which were designed as measures of learning, were submitted to a multiple factor analysis. The dogs included males and females of the following breeds: Basenji, Beagle, Cocker Spaniel, Shetland Sheep Dog, Wire-haired Fox Terrier and Basenji-Cocker Spaniel crosses. A centroid analysis of the intercorrelations among the 17 variables yielded five factors. Following orthogonal rotation of reference axes, the factors were interpreted as: activity and impulsiveness, docility or responsiveness to a human trainer, manipulation, visual observation, and persistence of positional habits. It is pointed out that one or more of these factors may reflect breed differences within the population investigated. An obverse factor analysis would further clarify breed differences. If data should eventually become available on sufficiently large numbers within each breed, separate factorial analyses for each breed would be desirable.

Some of the present findings indicate that tasks which may appear quite similar to the human experimenter often involve dissimilar factors for the animal. Moreover, the factorial composition of the same task may vary considerably at different stages of training, a fact which was suggested by the earlier results of Wherry (1939, 1940, 1941) on rats.

Another outstanding finding pertains to the predominance of bipolar factors. This, too, corroborates earlier factor analyses of animal behavior, and sharply contrasts with typical results on human abilities. In the present study, negative factor loadings are common and appear to be psychologically meaningful in terms of the proposed interpretation of the factors. Such a finding is probably related to the obvious intertwining of cognitive with emotional and motivational factors in animal behavior. On most of the learning tasks employed in the present study, the dogs' performance reflected emotional and motivational factors as much as, or more than, it reflected ability factors. As in the case of other factor analyses of animal behavior, the present findings thus suggest that the distinction between cognitive and non-cognitive aspects of behavior is not so sharply drawn in animals as in humans (cf. Anastasi, 1948). To what extent such a trait differentiation is the product of cultural influences in the human has not been determined. It is hoped that it will eventually prove feasible to conduct longitudinal studies on animals, whose object will be to alter the subjects' trait organization by controlled experiences. Such an approach should provide the answers to many questions regarding the nature and organization of psychological traits.

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4

The Chemical Nature of Holothurin, a Toxic Principle from the Sea-cucumber (Echinodermata: Holothurioidea)

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Introduction

A TOXIC principle in some Holothurioidea and its effects on fish and other small animals have been described in the literature (Saville-Kent, 1893; Frey, 1951). More recently, Nigrelli (1952) and Nigrelli & Zahl (1952) have reported on certain chemical and biological characteristics of a water soluble, heat stable substance isolated from the Bahamian[1] sea-cucumber, *Actinopyga agassizi* Selenka. This material, called Holothurin (Nigrelli, 1952), has been found to be toxic to a wide range of plants and animals, and also appears to have some anti-tumorous and other pharmacological properties[2].

Holothurin is a new variation on the theme of steroid compounds. It consists of several closely related aglycones or genins of steroid structure and a chain of several monosaccharides. It is soluble in water and produces a soapy foam in high dilution. Insofar as can be ascertained, this is the first known saponin of animal origin.

Chemical Studies

The starting material of these experiments consists of sun-dried Cuvierian glands of *Actinopyga agassizi*. It forms pinkish flakes and is almost completely soluble in water. The material is first extracted in 95% ethanol and the minor fraction is discarded; it is then dissolved in 50% ethanol, leaving behind cellular material and other debris. The resulting product, comprising 60% of the starting material, is neutral, practically free of nitrogen and exerts no reducing power for sugar reagents; it shows no absorption in the ultraviolet region of the spectrum and its specific rotation is about $[\alpha]_D^{25} = -19°$. Upon hydrolysis with normal hydrochloric acid for three hours, it yields about 60% of its weight as a mixture of water soluble reducing sugars and 40% as a mixture of water insoluble aglycones.

The sugar solution, when chromatographed on paper with butanol-ethanol-water at pH=3.7, appears to consist of three different sugars, the fastest moving of which is probably rhamnose with R_f=0.31. The next one with R_f=ca. 0.19 gives pentose reactions and is presumably identical with xylose, while the slowest one with R_f=0.14 is glucose and may be removed by fermentation with yeast. Their quantities are in the approximate ratio of 2:1:1. This mixture of sugars is reminiscent of the carbohydrate portion of cardiac glycosides and other saponins of plant origin.

The water insoluble moiety resulting from acid hydrolysis melts, after softening at 200°, over a wide range from 230° to 250°. This mixture of aglycones (1.0 g.) may be fractionated by repeated crystallization from acetone, ether and other organic solvents, and yields four fractions in the following quantities: 130 mg. genin A, 70 mg. genin B, 90 mg. genin C, and 420 mg. fraction F. These fractions melt respectively at 256-260°, 235-240°, and 195-220°; fraction F is an oily substance, soluble in petrolic ether and partially soluble in aqueous alkali. The optical rotations are as follows: substance A $[\alpha]_D^{25} = -27°$ (in ethanol), substance B $[\alpha]_D^{25} = -86°$, and substance C $[\alpha]_D^{25} = -124°$ (both in ethyl acetate). Fraction F presumably

[1]We wish to thank Dr. C. M. Breder, Jr., Director of the Lerner Marine Laboratory of the American Museum of Natural History, Bimini, B.W.I., for the use of the laboratory facilities in collecting the Holothurin material used in these studies.

[2]We wish to thank Dr. Sophie Jakowska, College of Mount Saint Vincent, New York City, and Mr. Herman Baker, Department of Chemistry, The Mount Sinai Hospital, for their assistance in testing the pharmacological action of some of the products derived in these studies.

consists of fatty acids and esters, and its weak optical rotation of $[\alpha]_D^{25}=-8°$ is probably due to contamination with some aglycone. Whether all of fraction F forms an intrinsic part of the molecule or is merely carried along by virtue of the detergency of the saponins is undecided. Substances A, B, C, as well as their acetyl derivatives, show ultraviolet absorption with $\lambda_{max}=$ 244 mμ and an inflection at 238 mμ. Extinction is highest, $E_{1cm}^{1\%}=$ 278, for acetyl-A, and $E_{1cm}^{1\%}=$ 194 for compound A itself. The acetyl derivative of fraction C shows a secondary maximum at $\lambda_{max}=$ 300 mμ with $E_{1cm}^{1\%}=$ 19.

On passing an aqueous solution of Holothurin prior to hydrolysis through a mixed bed ion-exchange resin column, all sodium and chloride ions may be removed. During this operation about 20% of the original solids, comprising electrolyte as well as much of the fatty material, remain on the column. The eluate is acidic; its optical rotation is $[\alpha]_D^{25}=-23°$. The toxicity of the material seems to be diminished by this operation. This product has no reducing power and yields on acid hydrolysis the water soluble sugars and about 33% of its weight of the mixed neutral aglycones. Optical rotation $[\alpha]_D^{25}=-77°$; elementary analysis: C, 72.27; H, 9.72; calculated for $C_{27}H_{44}O_5$ (average composition): C, 72.29; H, 9.88. M.p. 230-250°.

Fractionation of the aglycones yields a crystalline substance of m.p. 258-262° and $[\alpha]_D^{25}=$ −19°, which is identical with substance A above. Elementary analysis: C, 69.87; H, 9.06; calculated for $C_{27}H_{42}O_6$: C, 70.10; H, 9.15. A second fraction, melting at 228-230°, parallels fraction B above in its properties. Elementary analysis: C, 70.90; H, 9.09 and inorganic residue 4.24%. These observations indicate that the derived aglycones are closely related one to another and consist of a tetracyclic steroid skeleton with two conjugated double bonds and five to six oxygen functions mostly of hydroxyl nature.

Discussion

The carbohydrate components, presumably linked to a hydroxyl group on C_3, are easily hydrolyzed by dilute acid. At the same time a double bond (C:C or C:O) arises in conjugation with a previously present double bond. The wavelength of the resulting absorption maximum indicates that the two double bonds are situated in adjoining rings, e.g. in position C_4:C_5 (or C_8:C_{14}) and C_6:C_7. A highly substituted α:β-unsaturated ketonic structure cannot be excluded as an alternative. This unsaturated system may be independent of the glycosidic linkage, as it once arose on passage through the mixed bed column, where the glycoside portion of the molecule remains intact. The new double bond may be formed by saponification of an ester grouping in angular position and loss of the resulting free tertiary hydroxyl group by dehydration, or it may have arisen from the cleavage of an enol ether and concomitant migration of a double bond.

Summary

Holothurin, the toxic principle in the Cuvierian organ of *Actinopyga agassizi*, a Bahamian sea-cucumber, is a steroid saponin. Acid hydrolysis yields a mixture of levorotatory genins with a conjugated system of two double bonds and three sugars—rhamnose, xylose and glucose. Some of the chemical features of the aglycones are discussed.

This is the first known steroid saponin of animal origin.

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5

The Effects of Holothurin, a Steroid Saponin of Animal Origin, on Krebs-2 Ascites Tumors in Swiss Mice

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Introduction

THE preparation and general characteristics of the water soluble, thermostable factor, Holothurin, from the Cuvierian organ of the sea-cucumber, *Actinopyga agassizi* Selenka, have been described by Nigrelli (1952)[2] and by Nigrelli, Chanley, Kohn & Sobokta (1955). Nigrelli & Zahl (1952) reported that Holothurin inhibited growth of certain Protozoa and that *in vitro* treatment of Sarcoma 180 cells with this substance markedly reduced the subsequent growth of these tumor cells after inoculation into Swiss mice. The present preliminary report is primarily concerned with the *in vitro* and *in vivo* effects of Holothurin on the Krebs-2 ascites tumor in Swiss mice.

Materials and Methods

The Krebs-2 ascites tumor, carried in Swiss mice and in a breeding colony of Highline, ICR Swiss mice, was obtained in 1952 from Dr. T. S. Hauschka. The tumor has been maintained by weekly intraperitoneal inoculations of 0.1-0.2 ml. of ascitic fluid into 10-12 mice. All animals in these experiments were obtained from the original breeding mice by cousin matings, and weighed 18-25 grams at the beginning of the experiments.

The method recommended by Goldberg, Klein & Klein (1950) for counting the tumor cells and for the inoculation procedure was used. The donors of ascitic fluid had carried the tumor for at least 7 days but not more than 13 days. A uniform inoculum of 2×10^6 tumor cells suspended in sterile saline, as suggested by Sugiura (1953), was injected in all experimental animals. No remission has occurred in more than 2,000 mice inoculated with 1 to 44 $\times 10^6$ tumor cells.

Holothurin was dissolved in physiological saline and autoclaved for 30 minutes at 20 lbs. pressure. The method of Nigrelli & Zahl (1952) was used for treating the tumor cells *in vitro;* varying amounts of Holothurin were added to tubes containing 20×10^6 tumor cells diluted with sterile saline. The tubes were incubated at 99°F and shaken for 1 hour. Control tubes were similarly treated.

In the *in vivo* experiments 0.01, 0.05, 0.1 and 0.2 mg. of Holothurin were injected daily or spaced over 7-21 days. Repeated injections were made intraperitoneally on alternate sides of the abdomen. Controls with and without tumor cells were maintained simultaneously. Fifty-six mice were also inoculated with tumor cells in the subcutaneous areas of the axillary and inguinal regions. These did not survive for more than two and one-half months.

Results

Toxicity of Holothurin.—Intraperitoneal injection of 0.2 mg. of Holothurin in sterile saline was lethal in 48 hrs. for 6 female mice; injection of 0.1 mg. into 12 female mice caused no deaths. The safe upper limit for injection of Holothurin was taken as 0.1 mg.

In Vitro Experiments.—No deaths were observed in 60 days in female mice inoculated with 2×10^6 tumor cells treated with 0.1 mg. of Holothurin. These mice produced normal litters when mated; the offspring, however,

[1]This investigation was supported in part by grants C-1745 and C-1745-C from the National Cancer Institute of the National Institutes of Health, Public Health Service. We wish to thank Mr. Richard DiLorenzo and Mr. Paul Lachance for technical assistance.

[2]We wish to thank Dr. C. M. Breder, Jr., Director of the Lerner Marine Laboratory of the American Museum of Natural History, Bimini, B.W.I., for use of the laboratory facilities in the collection and preparation of Holothurin.

TABLE 1. STANDARDIZATION OF MEAN SURVIVAL TIMES AND BODY WEIGHT GAINS OF SWISS MICE CARRYING THE KREBS-2 ASCITES TUMOR*

No. of Mice	Sex	No. Mice/Cage	No. of Cages	Range of Mean Survival Time for 31 Cages	Mean of 21 Mean Survival Times
186	F	6	31	9.3-15.1 Days	11.9 Days

Range of Days of Death of 186 Mice	Mean Wt. of 186 Mice on Day of Inoculation	Range of Single Wts. of 186 Mice on Day of Inoculation	Range of 31 Mean Wts. of 31 Cages of Mice on 10th Day after Inoculation	Mean of 31 Mean Wts. on 10th Day after Inoculation
6-26	22.0 gm.	18-25 gm.	26-36 gm.	32 gm.

*All mice were inoculated with 2×10^5 tumor cells diluted with sterile saline. Saline controls not included.

TABLE 2. EFFECTS OF HOLOTHURIN ON SWISS MICE CARRYING AN INOCULUM OF 2×10^6 KREBS-2 TUMOR CELLS

No. of Mice	Sex	Mg. Holothurin Injected	No. of Holothurin Injections	Mean Survival Time (Days)*	Mean Wt. on 1st Day of Tumor Cell Inoculation	Mean Wt. on 10th Day after Inoculation
12	F	Controls	Controls	M 13.7 R 10-19	M 21.7 gm. R 18-25	M 30.2 gm. R 26-33
6	F	0.01 mg.	7 injections in 8 days	M 8.5 R 5-10	M 21.6 R 19-23	
6	F	0.05 mg.	7 injections in 8 days	M 19.5 R 14-28	M 20.0 R 18.5-21	M 23.2 R 19.3-25.5
6	F	0.10 mg.	7 injections in 16 days	M 23.5 R 5-35	M 22.7 R 19-25	M 20.6 R 16.7-25.2
6	F	0.10 mg.	10 injections in 21 days	M 34.6 R 8-51	M 21.9 R 19-25	M 20.6 R 18-22.5

*M is the mean day of death or mean weight of mice; R is the range of variation

TABLE 3. EFFECTS OF HOLOTHURIN ON SWISS MICE CARRYING AN INOCULUM OF 2×10^6 KREBS-2 ASCITES TUMOR CELLS

No. of Mice	Sex	Mg. Holothurin Injected	No. of Holothurin Injections	Mean Survival Time (Days)	Mean Wt. on 1st Day of Tumor Cell Inoculation	Mean Wt. on 10th Day after Inoculation
6	F	Controls	Controls	M 11.1 R 7-15	M 23.3 gm. R 21-24.5	M 29 gms. (7 days) R 23-33
24	F	0.1 mg.	10 injections in 20 days	M* 25.4 R 12-45	M 23.3 R 20-25	M 23.5 R 20.5-27.5

*The mean survival time is for 20 mice since 4 of the treated mice have lived for more than 6 months with no gross indication of a tumor.

showed no resistance to the tumor. In a control group of 6 mice, all were dead by the 18th day, with a mean survival time of 12.5 days.

Two deaths occurred in a group of 12 female mice that were given 2×10^6 tumor cells treated with 0.05 mg. of Holothurin. The amount of material needed to irreversibly inactivate 20×10^6 tumor cells at 99°F. for 1 hr. is between 0.05 and 0.1 mg.

In Vivo Experiments.—The efficacy of Holothurin as a tumor growth inhibitor was judged on the basis of a comparison of the body weight gain on the 10th day after tumor cell inoculation and of the mean survival time in treated and untreated mice.

A group of 186 female mice (31 cages of 6 mice/cage) was inoculated with the standard amount of tumor cells. All mice died between the 6th and 26th day after inoculation. The results, shown in Table 1, were used as the baseline for comparison with the individual controls and experimental mice. Another group of 120 animals gave similar results. Two mice in this group, however, survived past the 24th day, dying on the 33rd and 36th day after inoculation.

A series of 7 injections of 0.01 mg. of Holothurin started on the day after inoculation and continued for 7 days gave an apparent reduction of the mean survival time (Table 2). However, this mean survival time (8.5 days) falls very close to the lower limit of variation found among the 186 control mice (see Table 1). It is impossible, therefore, to conclude that there has been a true reduction in mean survival time with the amount of Holothurin used in this series.

A similar series of injections of 0.05 mg. of Holothurin caused a slight increase in mean survival time and a marked reduction in gains in body weight on the 10th day after inoculation (Table 2).

The daily injection of 0.1 mg. Holothurin was found to be lethal. However, a series of 7 injections given on alternate days resulted in some increase in mean survival time and a marked reduction of mean body weight on the 10th day. A series of 10 injections of 0.1 mg. spaced over 21 days produced a considerable increase in mean survival time and a very marked reduction of mean body weight gain on the 10th day after inoculation (Table 2).

The results from another group of 24 female mice inoculated with the standard amount of tumor cells and given a series of 10 injections of 0.1 mg. of Holothurin over a period of 20 days are shown in Table 3. The increase in survival time and the marked reduction in gain of mean body weight on the 10th day is clearly evident when compared to the experimental and

baseline controls. Four of the 24 treated mice showed no observable growth during a period lasting over 6 months.

Discussion

The unequivocal validity of a test based on 186 animals is, of course, open to question. The repetition of the test on 120 animals gave nearly identical values, but there were two control animals that survived past the 24th day after inoculation, dying on the 33rd and 36th day. There have been no remissions, however, in such inoculated animals, nor in more than 2,000 mice inoculated with Krebs-2 ascites tumor cells in amounts from 1 to 44 $\times$ 10^6.

The four remissions observed cannot be entirely explained by accidental subcutaneous inoculations. Of a group of 56 mice inoculated subcutaneously in the axillary and inguinal regions, none were living after two and one-half months.

Summary

Injection of 0.2 mg. of Holothurin in sterile saline into female Swiss mice (Highline, ICR) is lethal within 48 hrs.

The treatment of 20 $\times$ 10^6 Krebs-2 ascites tumor cells *in vitro* with 0.1 mg. of Holothurin by shaking such suspensions for 1 hr. at 99°F. results in inactivation of the tumor cells. The inoculation of such treated cells into normal mice produced no observable growth during a period of 60 days.

The daily injection of 0.01 mg. of Holothurin for 7 days, beginning the day after inoculation of the tumor cells, had no effect on the progressive growth of the tumor.

The daily injection of 0.05 mg. of Holothurin for 7 days, beginning the day after inoculation of tumor cells, brings about an increase in survival time and a marked reduction of mean body weight gain on the 10th day.

The spacing of one day between injections of 0.1 mg. of Holothurin in a series of 7-10 injections results in a marked increase in mean survival time and reduction in mean body weight gain on the 10th day after inoculation.

Four remissions of the tumor of more than 6 months duration occurred in a group of 24 mice treated with 0.1 mg. of Holothurin in a series of 10 injections over a period of 20 days.

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6

Regeneration of Melanomas in Fishes[1]

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(Plates I-IV; Text-figures 1-45)

COMPARATIVE pathologists who have studied the black pigment cell tumors of sharks, fishes, amphibians, reptiles, birds and mammals, have discovered that the melanocyte is the common cell type of the melanomas of vertebrate animals, including those of man (Gordon, 1951b). The living melanocytes from the melanomas of man, mouse and fish, when studied by tissue culture methods, have been shown to be similar morphologically (Grand, Gordon & Cameron, 1941, and Attardi & Moro, 1953). A comparative study of the enzyme reactions of melanomatous tissues from mammals and fishes also revealed that both are quite similar with respect to tyrosinase activity (Fitzpatrick, Lerner, Calkins & Summerson, 1950).

An outstanding peculiarity of the fish melanoma, in contrast with human melanoma, is the presence of macromelanophores. The large size of these melanophores and their capacity for drastic changes in the shape of their pseudopodial processes precludes their being confused with the much smaller melanocytes or the melanin-laden macrophages. From previous studies it was not possible to state whether macromelanophores and melanocytes were related ontogenetically, but it was known that melanomas arose in hybrid fishes only when macromelanophores were present.

Regeneration studies make possible the retracing of the progressive growth stages in the development of a melanoma. It will be shown that, in part, they confirm the observations of Gordon & Smith (1938) who demonstrated that in the earliest stages of this neoplasm, macromelanophores in their abnormal growth replace the normal tissues of the corium almost completely and create a state of melanosis. In later stages, Gordon & Smith showed that the large black pigment cells invade the deeper body areas, moving along the fascial tissues of the muscles. The macromelanophores eventually surround the muscle fibres and destroy them. Nodular lesions leading to the formation of melanomas usually arise in these primary zones of diffuse melanosis. During the early stages in the growth of the melanoma, the macromelanophores are the principal cells involved. As the growth of the melanoma progresses, the macromelanophores apparently redifferentiate into cells which have the morphological properties of melanocytes.

Study of these suspected cellular transformations of normal macromelanophores to malignant melanocytes, made possible by histological examination of the progressive growth of melanomas, obviously required additional study by more direct methods. Observations of regenerating tissues following the amputation of melanomas in the dorsal fin have made it possible to retrace the ontogenetic relationships between macromelanophores and melanocytes. By experiments that will be described, the close association of these pigment-forming cells has been confirmed. In an independent study, Marcus & Gordon (1954) followed the fate of melanoma fragments after they were transplanted into clear, transparent normal areas of the fish's skin. They, too, found that some melanocytes are capable of transforming into macromelanophores and vice versa.

MATERIAL AND METHODS

Fifty-three xiphophorin fishes were studied; they consisted of young and adult platyfish, *Xiphophorus maculatus*, and platyfish-swordtail

[1]Supported by grants from the National Cancer Institute of the National Institutes of Health, U. S. Public Health Service, and the Anna Fuller Fund. The work was aided by the laboratory facilities of the American Museum of Natural History, New York 24, New York.

hybrids, *X. maculatus–X. helleri.* Their histories are listed in Table 1.

The fishes chosen for the study of the effects of amputation of normally and atypically pigmented dorsal fins were obtained from genetically known stocks maintained at the Genetics Laboratory of the Aquarium, New York Zoological Society, by methods described by Gordon (1950a). The most important pigmentary patterns for these studies were those that are produced in the dorsal fins by two types of specialized pigment-forming cells, the micromelanophores and macromelanophores. Various combinations of these two kinds of pigment effector cells in the dorsal fins of fishes were available, so that it was possible to compare the results of amputation and regeneration of normally pigmented dorsal fins with those that were either in a state of melanosis or had melanomas. In those that had melanomas, it was also possible to compare those that had the typical black coloring with those that had amelanotic melanomas.

The fishes were first anesthetized with a 1:2000 solution of MS 222 (Triacine Methanesulfonate produced by Sandoz) and placed on a wet cotton pad. The dorsal fins were removed close to the body, using iridectomy scissors. The regenerating fins were observed at intervals under the binocular microscope and drawings were made of the progressive regrowths.

For histological study, tissues amputated and regenerated were fixed in Bouin's solution, decalcified in nitric acid-phloroglucin, dehydrated in dioxane, embedded in paraffin and sectioned at 8 to 10 micra. Sections were studied either with Mayer's hematoxylin (hemalum) and eosin, or with a modification of the Masson's trichrome stain. In order to obtain the cellular details of extremely black tumors, the sections were first treated with celloidin and then bleached in a solution of potassium chlorate crystals in hydrochloric acid and 70% alcohol.

Regeneration of Dorsal Fins in Platyfish

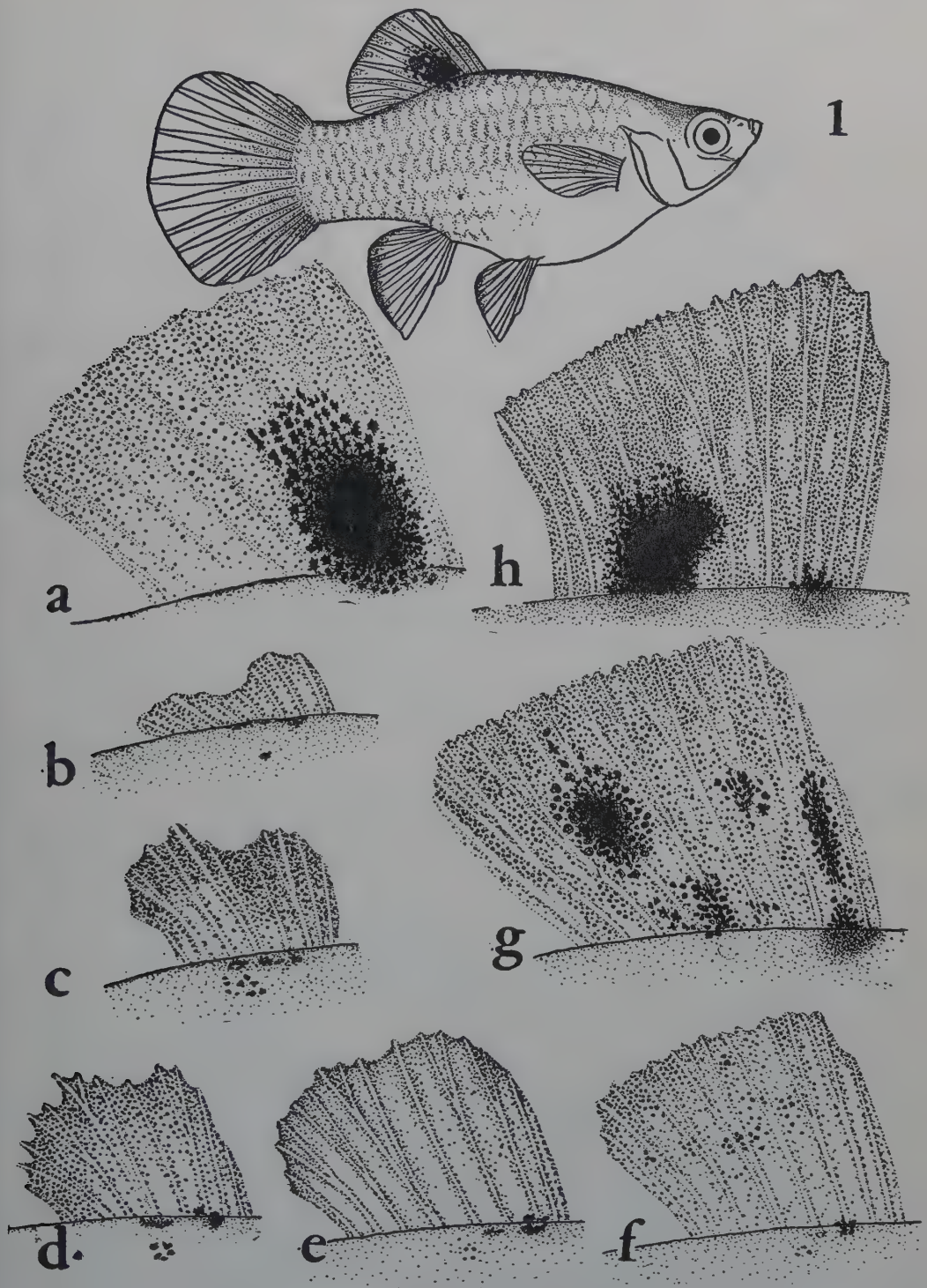
The spotted dorsal fins of two female and three male platyfish were amputated, Table 1, No. 1. Each fin had several groups of normal macromelanophores as well as many micromelanophores, Text-fig. 1. Within 24 hours wound healing began and within five to seven days the blastema, about 0.5 mm. in height, was formed. The blastema contained some pigment granules and the stumps of the fin rays, each of which was composed of two lepidotrichial elements. Within two weeks the blastema grew to about 1 to 1.5 mm. in height. Micromelanophores appeared along the regenerating fin rays, in the fin membranes and on the fin's distal edge, Text-fig. 1b. Within one to one and a half months the fin grew to 2 to 3 mm., Text-figs. 1c, 1d. In two animals some macromelanophores migrated into the base of the regenerating fin from the region ventral to the dorsal fin, Text-fig. 1d. The fin rays began to bifurcate at their tips. At three to four months in four out of five fish, the new dorsal fin reached its original size, 5 to 6 mm. in height. Only eight fin rays regenerated in one fish to replace the original 10, Text-fig. 1f. In one animal originally having several spots on its dorsal, within eight months, the macromelanophores formed one big spot at the posterior base of the regenerated fin, Text-figs. 1g, 1h. In another fish the large spots did not form at all. Usually macromelanophores appeared in the

Table 1. Genetic Histories of Fishes Used in Regeneration Studies of Normal and Atypical Pigment Cell Growths in the Dorsal Fins[1]

Group	No.	Pedigree	Genetic Constitution	Melanophores
1.	5	163	*Sd* + + Spotted-dorsal	Micro. + Macro.
2.	2	306	+ *Co* + Comet	Micro.
3.	3	306	+ *Co E* Wagtail	Micro.
4.	5	306	*Sd Co* + Spotted-dorsal, Comet	Micro. + Macro.
5.	3	306	*Sd Co E* Spotted-dorsal, Wagtail	Micro. + Macro.
6.	2	311, 316	+ + Unpatterned	Micro.
7.	18	311, 316	*Sd* + Melanosis, variable (1, 2, 3)[2]	Micro. + Macro.
8.	10	311, 316	*Sd* + Melanomas	Micro. + Macro.
9.	5	311, 316	*Sd i* Amelanotic melanoma	Not evident

[1]Summary of 53 histories. Groups 1 to 5 represent *Xiphophorus maculatus,* 6 to 9 represent *X. maculatus–X. helleri* hybrids. The individual records are presented in Tables 2 to 6.

[2]The degree of melanosis is indicated arbitrarily: 1, severe; 2, intermediate; 3, slight; see text for criteria.



TEXT-FIG. 1. Regeneration of the spotted dorsal fin of a platyfish, genetically *Sd co e*. Macromelanophores produce the spotted pattern in the dorsal fin. Micromelanophores are also present in the fins and on the body. 10 ×. **a**—Before amputation. **b**—Within 13 days following amputation. **c**—Within 40 days. **d**—Within 63 days. **e**—Within 90 days. **f**—Within 120 days. **g**—Dorsal fin of another fish of the same stock before amputation. **h**—The regenerate of the fin shown in figure g within 8 months following amputation.

TABLE 2. REGENERATION, AFTER AMPUTATION, OF DORSAL FINS AND THE SPOTTED PATTERNS IN PLATYFISH[1]

No.	Pattern	In days	Regeneration Of Fin	Regeneration Of Pigmentation
1.	Spotted-dorsal	240	Complete	Similar
2.	Spotted-dorsal	240	Complete	Lacking
3.	Spotted-dorsal	229	Complete	Lacking
4.	Spotted-dorsal	188	Complete	Lacking
5.	Spotted-dorsal	62	Complete	Lacking

[1]*Xiphophorus maculatus* of pedigree number 163, see Table 1, item 1, for genetic constitution.

regenerated tissues, if at all, much later than the micromelanophores.

The dorsal fins of two comet platyfish with micromelanophores only, Table 1, No. 2, were amputated for purpose of comparison. The comet pattern is made up of closely grouped micromelanophores and is confined to the caudal fin only, Text-fig. 2. Within a week a blastema developed, 0.3 to 0.5 mm. in height, which contained fin rays and some micromelanophores at the base, Text-fig. 2b. The number of micromelanophores increased along the fin rays and they covered the base of the fin, Text-fig. 2c; they reached the distal edge of the new fin in two weeks. Within a month, the height of the regenerate was 3.5 to 4 mm., and some of the regenerating fin rays had bifurcated, Text-fig. 2d. Within two to three months the new fin reached its original height, 5 to 6 mm., and pigmentary pattern, Text-fig. 2e. Within four to seven months there were no further appreciable changes except for the secondary bifurcation of some fin rays.

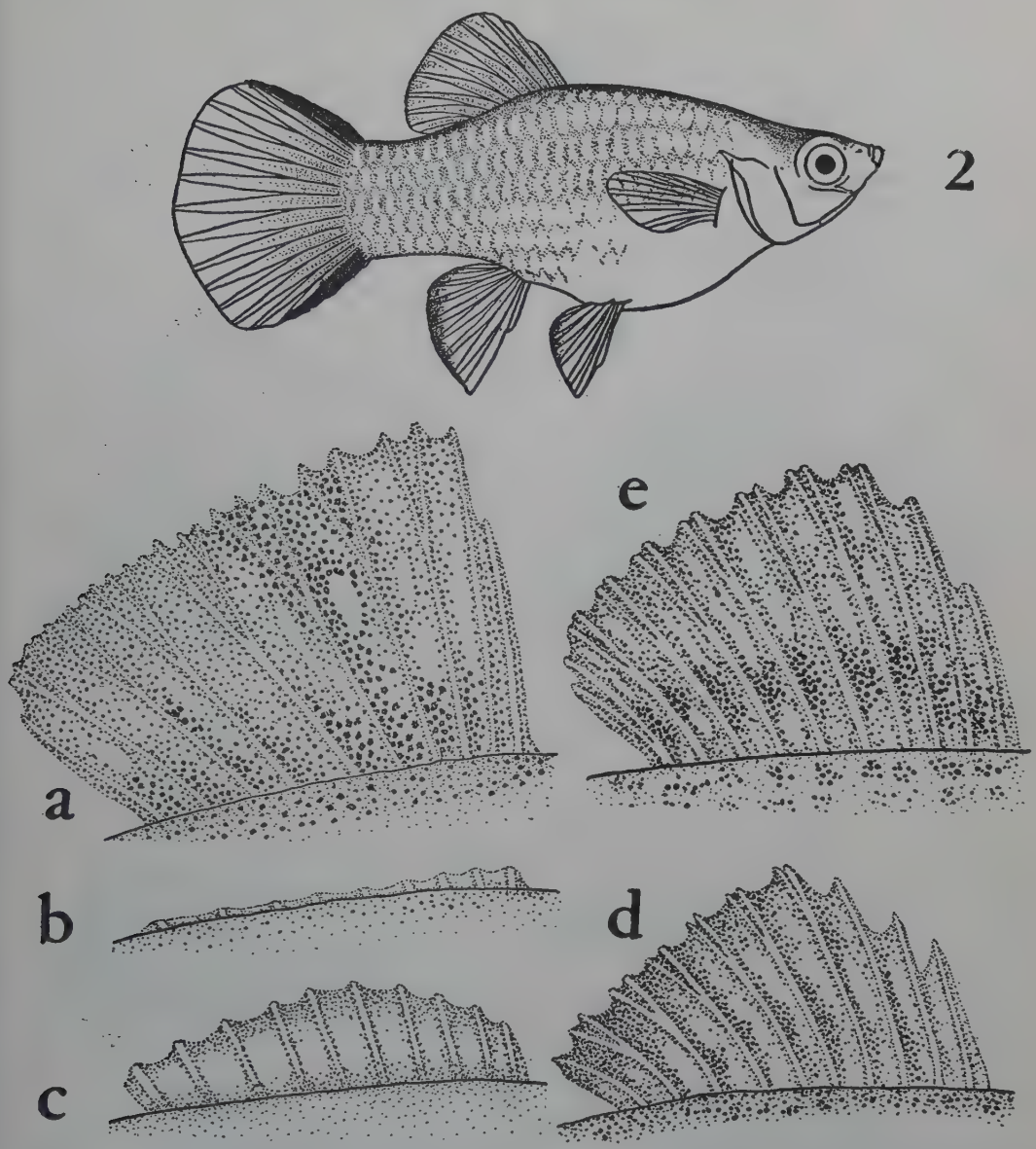
The dorsal fins of three platyfish with the wagtail pattern were amputated next, Table 1, No. 3. The dorsal fins of the wagtail platyfish had micromelanophores only, but they were much more numerous in all the fins than in the fins of the comet platyfish. The fins of the wagtail regenerated normally; however, the rate of the micromelanophore restoration and the number of regenerating micromelanophores were greater than in the comet platyfish.

The heavily spotted dorsal fins of five platyfish were amputated; they had both micro- and macromelanophores in the dorsal fin and a comet pattern in the tail, Table 1, No. 4. One specimen had macromelanophores that had spread down from the dorsal fin to both sides of the body. There they had infiltrated the underlying tissues, creating a condition of melanosis. Within a week following amputation of the dorsal fins, blas-

TABLE 3. REGENERATION, AFTER AMPUTATION, OF DORSAL FINS AND PIGMENTARY PATTERNS IN PLATYFISH[1]

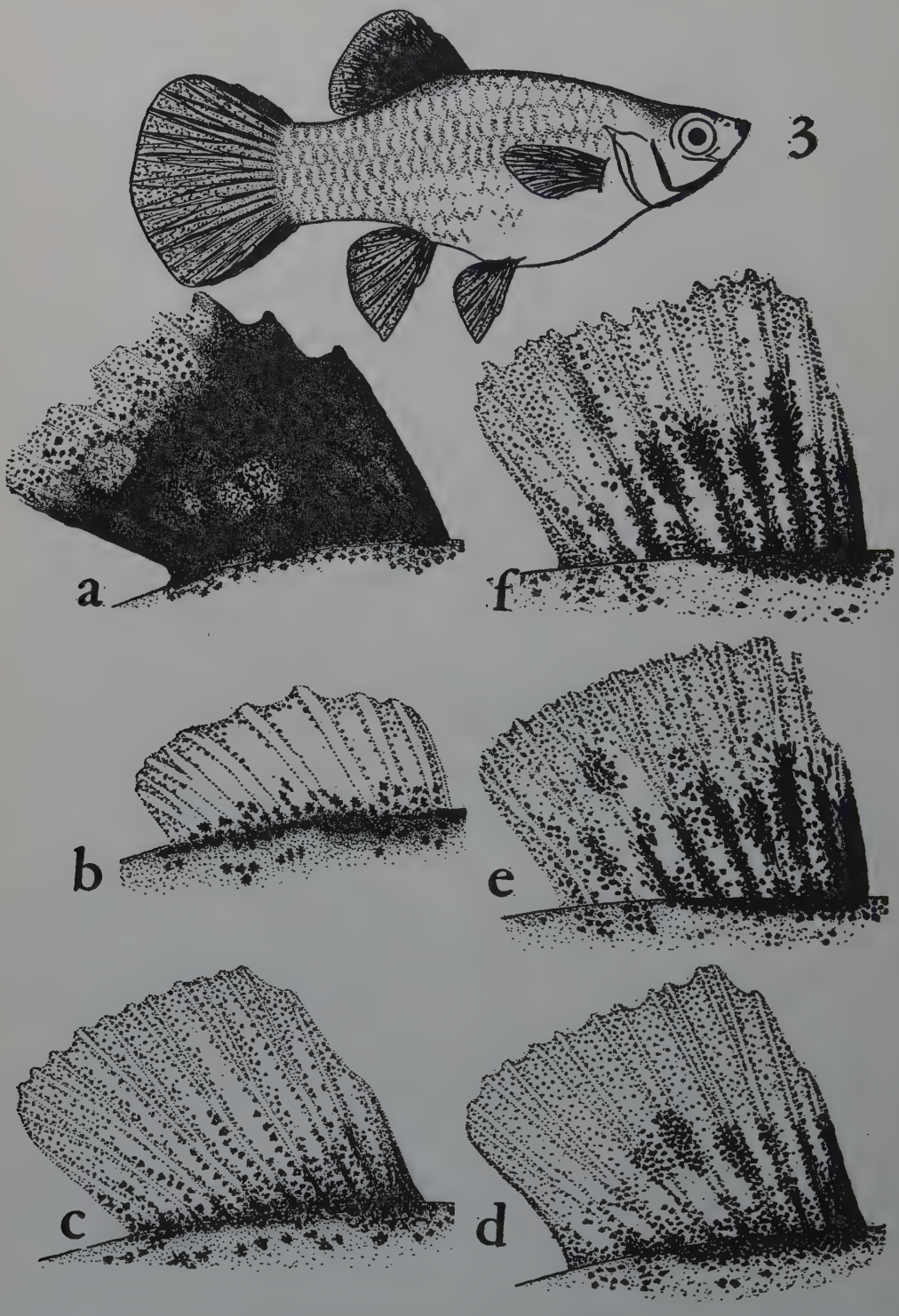
No.	Pattern	In days	Regeneration Of Fin	Regeneration Of Pigmentation
6.	Comet[2]	233	Complete	Complete
7.	Without Comet	23	Incomplete	Fixed[3]
8.	Wagtail[2]	190	Complete	Complete
9.	Wagtail[2]	7	Incomplete	Fixed[3]
10.	Wagtail[2]	2	Incomplete	Fixed[3]
11.	Spotted-dorsal, Comet	180	Complete	Incomplete
12.	Spotted-dorsal, Comet	90	Incomplete	Incomplete
13.	Spotted-dorsal, Comet	1	Incomplete	Fixed[3]
14.	Spotted-dorsal, Comet	21	Incomplete	Incomplete
15.	Spotted-dorsal, Comet	15	Incomplete	Fixed[3]
16.	Spotted-dorsal, Wagtail	180	Complete	Incomplete
17.	Spotted-dorsal, Wagtail	104	Complete	Incomplete
18.	Spotted-dorsal, Wagtail	55	Complete	Incomplete

[1]*Xiphophorus maculatus* of pedigree number 306, see Table 1, items 2, 3, 4, 5.
[2]Comet and Wagtail patterns are made up of micromelanophores only.
[3]Sacrificed for histological study.



TEXT-FIG. 2. Regeneration of the unspotted dorsal fin in a comet platyfish, genetically *sd Co e*. No macromelanophores are present in this specimen. 10 ×. **a**—Before amputation. **b**—Within 1 week following amputation. **c**—Within 2 weeks. **d**—Within 30 days. **e**—Within 60 days.

temas developed that were 0.3 to 0.5 mm. and contained some pigment particles and fin ray stumps. Within two weeks the blastemas were 0.5 to 1 mm. in height and contained many micromelanophores and four to five macromelanophores that had migrated from the base of the fin. Within two to three months the new fins of four of the platyfish were as high as the originals; one of them regenerated in slightly more than three months. Within two months the macromelanophores in the fins reformed the typical spotted dorsal pattern. Almost no micromelanophores were found in the spotted region, whereas many of them were present in the distal region of the fin. As time went on, more macromelanophores continued to migrate into the new fins, increasing the size of the spots, but even at six months the size of the macromelanophore spottings in the dorsal fin was not as large as in the originals.



TEXT-FIG. 3. Regeneration of spotted dorsal fin of a wagtail platyfish, genetically *Sd Co E*. This type of platyfish has many more micromelanophores in its fins than the *Sd co e* fish shown in Text-figure 1. 10 ×. **a**—Before amputation. **b**—Within 10 days following amputation. **c**—Within 33 days. **d**—Within 62 days. **e**—Within 120 days. **f**—Within 150 days.

The heavily spotted dorsal fins of three wagtail platyfish, Table 1, No. 5, were amputated. A strong melanosis produced by both micro- and macromelanophores existed in the dorsal fins Text-fig. 3a. Within a week a blastema formed, 0.5 to 1 mm. in height, which contained pigment particles and stumps of the fin rays. After 10 days the fin rays had begun to regenerate in the normal way and the micromelanophores reached the distal edge of the fin, producing a "Pigmentsaum" effect, a term used by Bösenberg (1938); during the same period macromelanophores also migrated into the fin from its base, Text-fig. 3b. After two weeks the regenerated fin was 1.5 mm. in height. Within a month it was 3 to 4 mm. and the micro- and macromelanophores had increased in number and the latter had begun to form black spots at the base of the fin, Text-fig. 3c. The macromelanophores had migrated over and along the fin rays; as a consequence, the basal part of the rays was completely covered. Within two to three months the new fin attained its previous size, 4.5 to 5 mm. in height. The macromelanophores continued to invade the regenerated fin, Text-figs. 3d to f. But even six months after the amputation the melanosis was not as strong as it was in the original fin, Text-fig. 3g. It appears, then, that after the fins regenerate normally they are invaded by macromelanophores which produce a somewhat lesser state of melanosis.

Regeneration of the Dorsal Fins in Species Hybrids between the Platyfish and Swordtail

Some spotted-dorsal hybrids exhibited melanosis, others typical melanomas, while still others showed amelanotic melanomas in their dorsal fins. For purposes of comparison, however, two platyfish-swordtail hybrids without macromelanophores but with micromelanophores in the dorsal fins were first studied, Table 1, No. 6. Within one week after amputation, blastemas 0.2 to 0.5 mm. were formed. Within two weeks the blastemas were 1 to 1.5 mm. and contained fin rays. Micromelanophores soon reached the distal edge of the regenerating fins, forming pigmented borders. The pigment cells migrated along both sides of the fin rays, leaving clear areas between the rays. Within two months the fin rays began to bifurcate. Within three months the fins reached their previous size of 5 to 6 mm.; their former pigmentary patterns were at that time restored.

The regeneration process was observed in eighteen platyfish-swordtail hybrids with dorsal fin melanosis, Table 1, No. 7. In some hybrid fish the melanosis was strong; in others just a few discrete macromelanophores were present in the dorsal fins. Most hybrids had, in addition to a melanosis of the dorsal fin, a melanosis of the tail and body, especially in the region under the dorsal fin, Text-fig. 4.

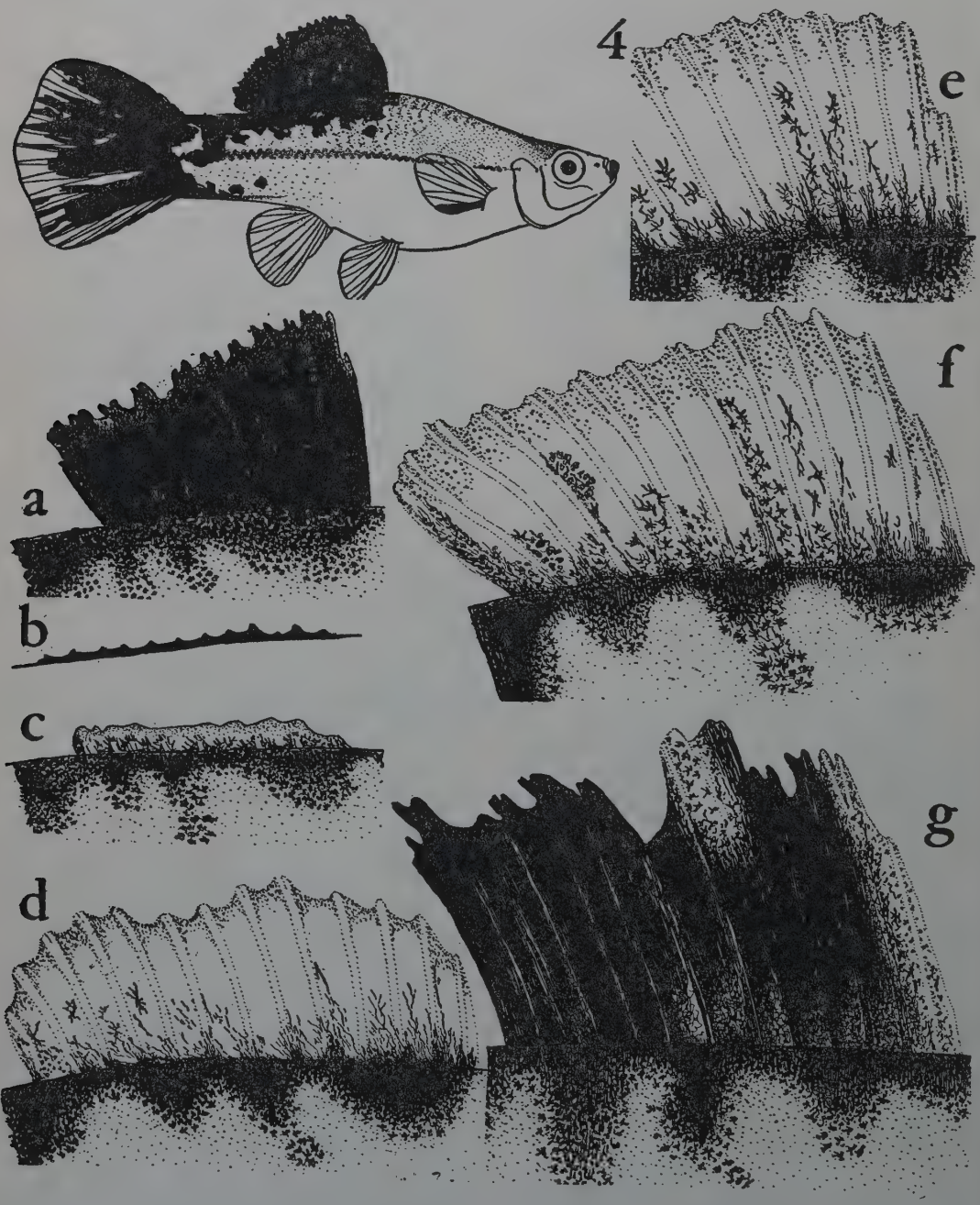
After amputation of the dorsal fins of fish with incomplete melanosis, blastemas containing some pigment particles were formed within five to seven days. Within 10 to 15 days the regenerated fins were 1 to 1.5 mm. and contained micromelanophores and fin rays. Macromelanophores began to appear at the base of the regenerating fins within 14 days in those that had been highly melanotic, and within 25 to 30 days in others. Within about three months the regenerated fins reached their original size, 8 to 10 mm. The new pigment patterns produced by the macromelanophores in the dorsal fins were not always the same, nor was the degree of melanosis as great as in the originals.

In those fish showing strong melanosis in their dorsal fins, the regeneration of the macromelanophores was more rapid; one, Text-figs. 4 to 4g, will be described in detail. During the healing of the wound, the epidermis contained dense pigment particles derived from the degenerating melanophores; this cleared up within one week. Dendritic processes of the macromelanophores, located just below the removed fin, penetrated the blastema and later whole cells entered it, Text-figs. 4b, 4c. Within two weeks the regenerated fin was 3 mm. and had many micromelanophores in its distal part; discrete macromelanophores appeared between the rays and formed a melanosis at the base of the fin, Text-fig. 4d. The fin reached its full height within two months, Text-figs. 4e to 4g, during which time melanosis was intensified. The 8½-months-old regenerated fin was almost as melanotic as the original fin, Text-fig. 4g.

The migration paths of the macromelanophores from their prior positions on the body just ventral to the fin into the regenerated fin between rays 7 and 8 may be seen in Text-fig. 4h. Distally some discrete macromelanophores contain fine grayish melanin granules that represent newly formed macromelanophores; these have been transformed from melanocytes. Within two months the previously produced darker macromelanophores that had originated from the base of the fin, and the newly formed gray macromelanophores, came together and produced a state of melanosis, Text-figs. 4i, 4j. Thus, there are two sources of macromelanophores in the regenerate: from those that were already present below the fin and from macromelanophores that are formed *in situ* from melanocytes in the regenerate itself.

The development of melanosis was faster in young hybrids than in mature ones. For ex-

ample, in young animals of 16 to 20 mm. in standard length with complete melanosis in the dorsal fin, as well as in the body below the dorsal fin, the macromelanophores migrated into the blastema within four days after amputation. Within three weeks the large black pigment cells reproduced an almost complete melanosis. The regeneration of the dorsal fin of one young fish of 21 mm. in length was peculiar. The anterior part of its original fin and the



TEXT-FIG. 4. Regeneration of the spotted dorsal fin of a platyfish-swordtail hybrid with severe melanosis in the dorsal fin. a to g, 5×. **a**—Before amputation. **b**—Within 2 days after amputation. **c**—Within 7 days. **d**—Within 14 days. **e**—Within 21 days. **f**—Within 30 days. **g**—Within 250 days; note that melanosis is almost the same as it was in the uncut fin, figure a.

TABLE 4. REGENERATION, AFTER AMPUTATION, OF DORSAL FINS AND PIGMENTATION IN PLATYFISH-SWORDTAIL HYBRIDS[1]

No.	Pattern	In days	Regeneration Of Fin	Regeneration Of Pigmentation
19.	Micromelanophores[2]	165	Complete	Complete
20.	Micromelanophores[2]	120	Complete	Complete
21.	Melanosis (3)[3]	257	Complete	Melanosis (2)
22.	Melanosis (2)	196	Complete	Melanosis (1)
23.	Melanosis (2)	180	Complete	Melanosis (2)
24.	Melanosis (2)	25	Incomplete	Fixed
25y.[4]	Melanosis (3)	30	Incomplete	Melanosis (3)
26.	Melanosis (2)	1	Incomplete	Fixed
27.	Melanosis (3)	1	Incomplete	Fixed
28y.	Melanosis (3)	21	Complete	Melanosis (3)
29y.	Melanosis (3)	6	Incomplete	Fixed
30.	Melanosis (3)	27	Incomplete	Fixed
31.	Melanosis (3)	30	Incomplete	Melanosis (2)
32.	Melanosis (3)	4	Incomplete	Fixed
33.	Melanosis (3)	210	Complete	Melanosis (3)
34y.	Melanosis (2)	30	Bilobed	Melanosis (1)
35y.	Melanosis (2)	30	Complete	Melanosis (1)
36.	Melanosis (2)	30	Incomplete	Melanosis (1)
37.	Melanosis (2)	9	Incomplete	Fixed
38.	Melanosis (2)	280	Complete	Melanosis (1)

[1] *Xiphophorus maculatus–Xiphophorus helleri* hybrids, pedigree numbers 311 and 316, see Table 1, items 6, 7.
[2] Fish numbered 19 and 20 had no macromelanophore patterns in dorsal fins; used for controls.
[3] The severity of melanosis is indicated by numbers in parentheses.
[4] y represents an immature specimen.

TEXT-FIG. 4 (*Continued*). h–The proximal area of the regenerate between 7th and 8th fin rays as seen under higher magnification, after 30 days. The macromelanophores are the dark cells which had moved up from the base of the fin. The melanocytes are the gray cells. These contain fine, dispersed melanin granules. i–Same after 38 days. Newly formed macromelanophores and melanocytes are located between the rays. j–After 60 days. The growth of macromelanophores has created a state of melanosis.

TABLE 5. REGENERATION, AFTER AMPUTATION, OF MELANOMAS IN THE DORSAL FINS OF PLATYFISH-SWORDTAIL HYBRIDS[1]

No.	Pattern	Regeneration In days	Regeneration Of Fin	Regeneration Of Melanoma
39.	Melanoma on and below fin	75	Bilobed	Melanoma
40.	Melanoma	300	Bilobed	Melanoma
41.	Melanoma	180	Bilobed	Melanosis (3)
42.	Melanoma	346	Complete	Melanoma
43.	Melanoma	69	Bilobed	Melanosis (3)
44.	Melanoma on and below fin	46	Complete	Melanosis (3)
45.	Melanoma on and below fin	1	Incomplete	Fixed
46.	Melanoma on and below fin	20	Incomplete	Fixed
47.	Melanoma	198	Complete	Melanosis (3)
48.	Melanoma	13	Incomplete	Fixed

[1]*Xiphophorus maculatus-helleri* hybrids of pedigree number 311, see Table 1, item 8.

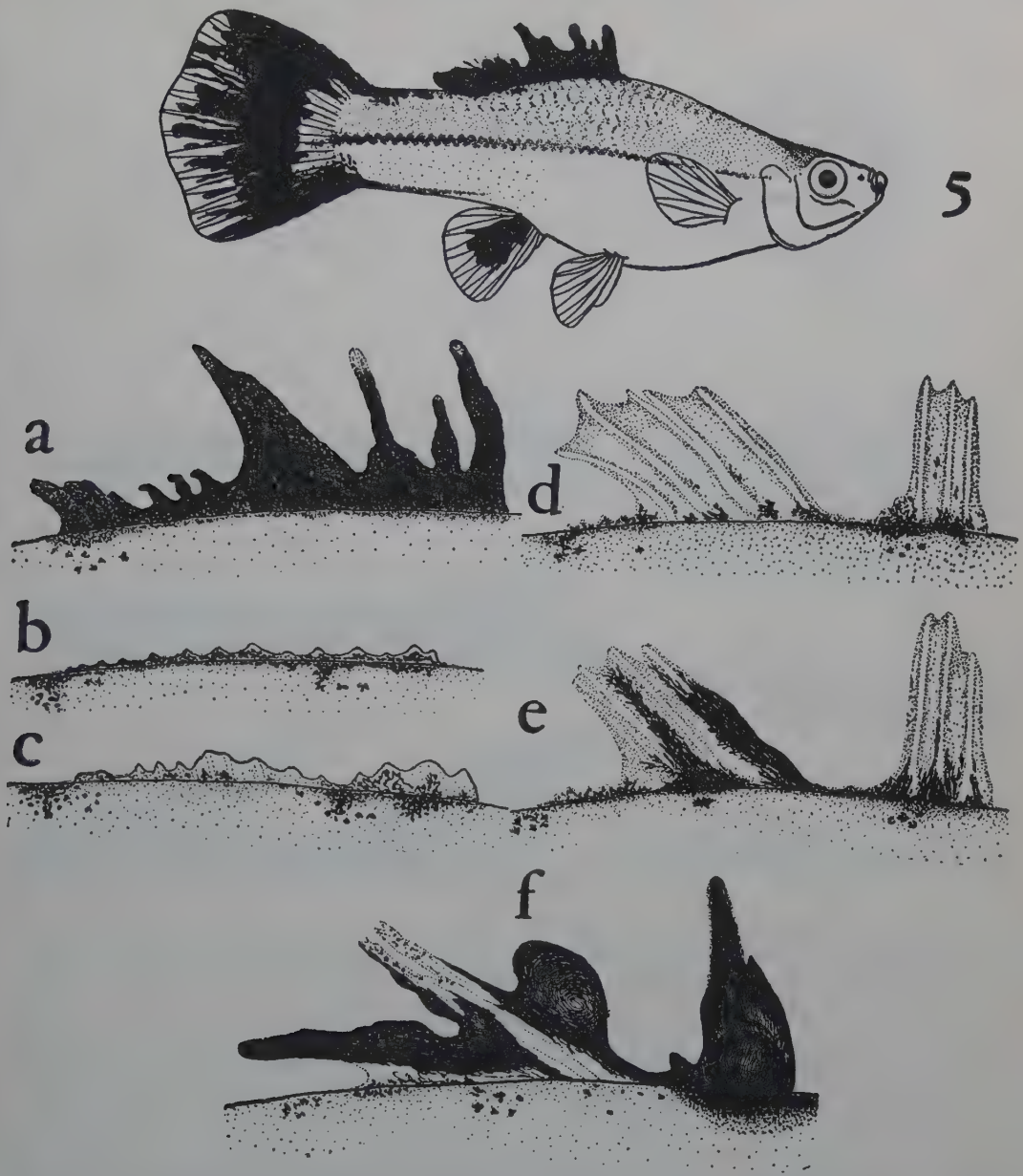
body just below it were entirely melanotic, whereas the posterior part of the fin and the body below it were not. After amputation the middle part of the fin did not regenerate. Within a month the anterior part of the new fin grew to 3 mm. and showed an almost complete melanosis, whereas the posterior part, which was 2 mm., showed a much lesser degree of melanosis. These observations suggest that when the tissues underlying the removed fin are completely melanotic at the time of operation, the melanosis in the regenerated fin develops more rapidly and becomes complete.

The regenerations of fins in 10 platyfish-swordtail hybrids with melanotic melanomas in their dorsal fins were observed, Table 1, No. 8. In some of these hybrids the dorsal fins were destroyed to various degrees, and in others secondary melanomas had developed on various parts of their bodies, Text-figs. 5 and 6.

In hybrids that had melanomas in the dorsal fin only, the regenerated fins were usually abnormal. Within a week after a melanomatous fin was amputated, a blastema formed which contained much pigment cell debris, Text-figs. 5a, 5b. Within one month the anterior and posterior parts of the fin regenerated but not the middle, Text-figs. 5c, 5d. After two months the regenerated fin became melanotic, Text-fig. 5e. Within 10 months a melanoma formed at the base of the anterior part of the fin, and a second nodular melanoma developed on the posterior part, Text-fig. 5f. In another animal, within six months following amputation, the regenerated fin was in a state of melanosis; it was then fixed for the histological study. After amputation of a destructive, bilobed, melanotic melanoma in the dorsal fin of a third hybrid, a non-tumorous, bilobal, deeply pigmented fin regenerated within 11 months. It measured 7.5 mm., whereas the removed fin had been only 1.5 mm. posteriorly and 5.5 mm. anteriorly.

In hybrids that had melanoma both in the dorsal fin and on the body ventral to it, the redevelopment of tumors in the regenerated fins was more rapid. For instance, one hybrid had a melanoma of the dorsal fin as large as the fin itself, and had infiltrated the tissue just below the fin, Text-fig. 6. After the fin together with the growth had been amputated, a melanotic bilobed fin developed at first, Text-figs. 6b to 6e, and then after 75 days a melanoma developed at the base of the anterior lobe and another around the posterior part, Text-fig. 6f. These observations suggest that when the melanoma is restricted only to the dorsal fin, the period of redevelopment of the tumor in the regenerated fin is longer than in those fish that exhibit melanoma both on the dorsal fin and on the body below that fin.

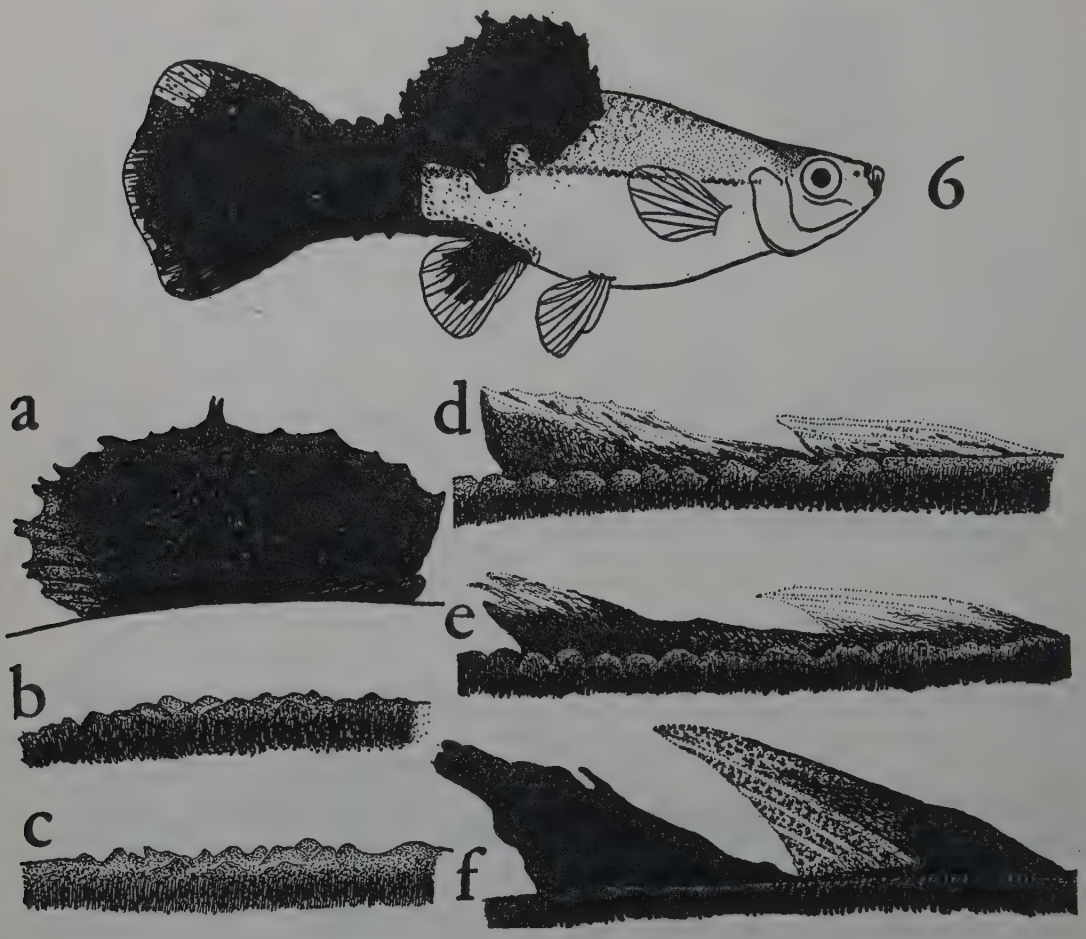
The fins of five platyfish-swordtail albino hybrids that had *amelanotic melanomas* in their dorsal fins were amputated, Table 1, No. 9. The melanomas were heavy and pink-colored; they had partly invaded the bodies below the dorsal fins. Each fish that had its melanomatous dorsal fin amputated reacted differently, but the responses were only slightly different from those



TEXT-FIG. 5. Regeneration of melanomas in the dorsal fin of a platyfish-swordtail hybrid. 5×. **a**—Before amputation. **b**—Within 7 days following amputation. **c**—Within 14 days. **d**—Within 30 days. **e**—Within 60 days. **f**—Within 10 months.

of the fish having typical melanomas that were previously described.

In one albino hybrid the original tumor was nodular and restricted to the anterior part of the fin. This part of the fin was either surrounded or destroyed by the tumor; the posterior part was also tumorous but the fin rays were still visible. The entire tumor of the dorsal fin contained large branching blood vessels clearly visible near its surface. Within a week following amputation, a 0.6 mm. blastema formed which contained no visible blood vessels or fin rays. At two weeks the regenerated fin measured 1 mm.; within one month it was 2 mm. and had five visible fin rays on its distal edge. Apparently the blastema and the tumor developed simultaneously, because within two months the basal one-third of the regenerated fin had a heavy melanoma; the re-

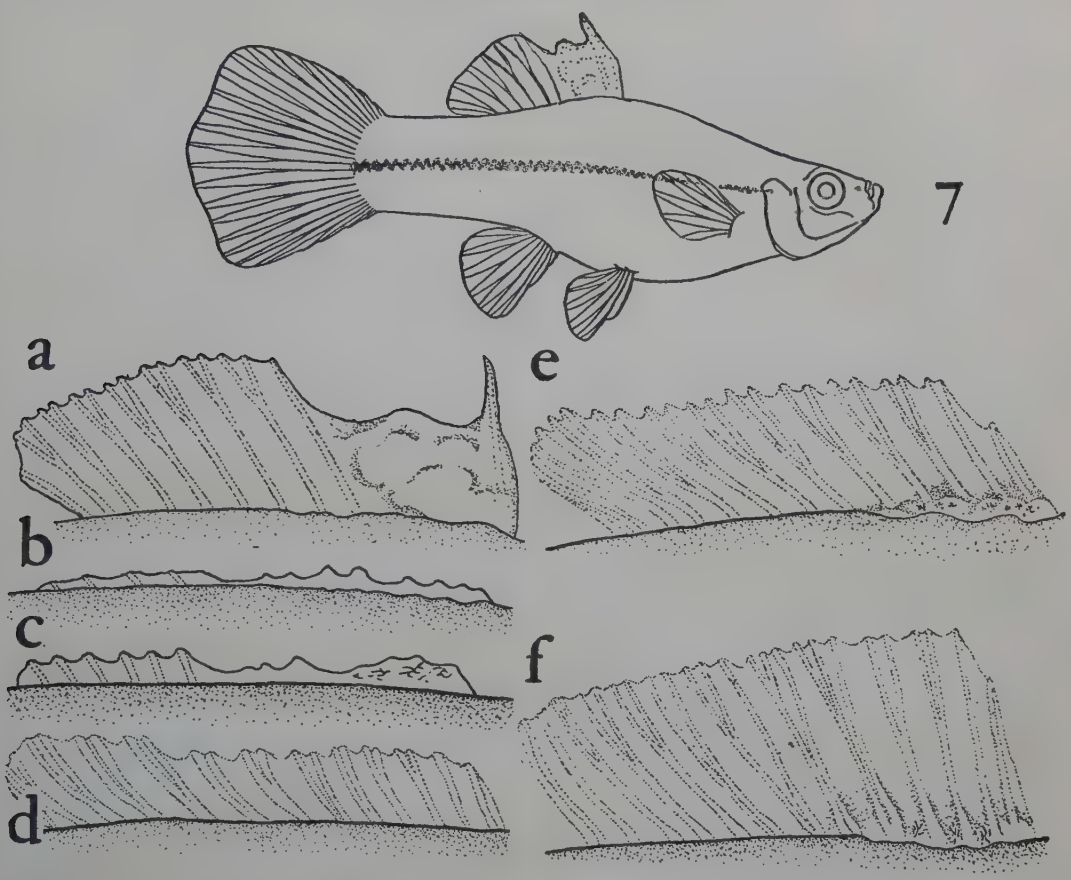


TEXT-FIG. 6. Regeneration of a melanoma in the dorsal fin of a platyfish-swordtail hybrid in which the melanoma had extended into the body ventral to the fin. 10 ×. **a**—Before amputation. **b**—Within 7 days following amputation. **c**—Within 14 days. **d**—Within 21 days. **e**—Within 30 days. **f**—Within 45 days.

TABLE 6. REGENERATION, AFTER AMPUTATION, OF AMELANOTIC MELANOMAS IN THE DORSAL FINS OF PLATYFISH-SWORDTAIL HYBRIDS[1]

No.	Pattern	In days	Regeneration Of Fin	Regeneration Of Melanoma
49.	Amelanotic melanoma	90	Complete	Amelanotic melanoma at base of fin
50.	Amelanotic melanoma on, below fin	90	Complete	Amelanotic melanoma
51.	Amelanotic melanoma on, below fin	60	Complete	Amelanotic melanoma
52.	Amelanotic melanoma on, below fin	20	Incomplete	Fixed
53.	Amelanotic melanoma on, below fin	30	Incomplete	Amelanotic melanoma

[1]*Xiphophorus maculatus-helleri* hybrids of pedigree 311, see Table 1, item 9.



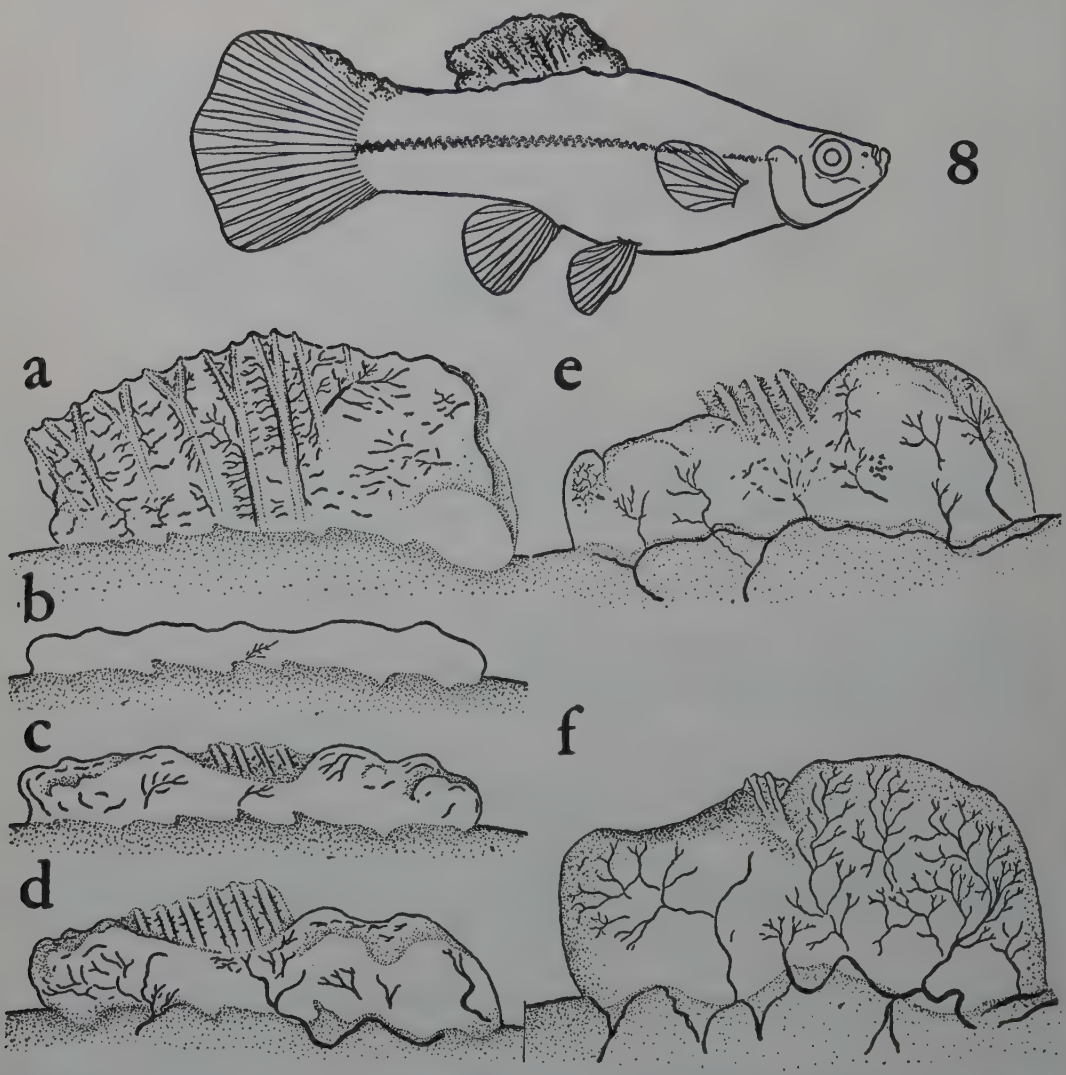
TEXT-FIG. 7. Regeneration of an amelanotic melanoma in the dorsal fin of a platyfish-swordtail hybrid. 6 ×. **a**—Before amputation. **b**—Within 1 week following amputation. **c**—Within 17 days. **d**—Within 1 month. **e**—Within 2 months. **f**—Within 3 months.

mainder was normal and contained 12 rays. All parts of the new growth were highly vascularized, but its general appearance was entirely different from that of the original fin. The fish died two months after its fin was amputated.

In another hybrid, an amelanotic melanoma in the anterior region of a 10-rayed dorsal fin was amputated, Text-fig. 7. A blastema containing four fin ray stumps developed within a week, Text-fig. 7b. Within two weeks the regenerated fin grew to 1 mm. and contained six fin rays, Text-fig. 7c. Within a month it was 3 mm. and had 13 rays, three more than the original one, Text-fig. 7d. A melanoma had developed at its former site, at the base of the anterior region of the fin, Text-fig. 7e. Within three months the regenerated fin reached its previous height of 5 mm., at which time the fish was fixed for histological study.

Another albino had a heavy, highly vascularized melanoma in its dorsal fin in which some fin rays were visible, particularly their tips. The tumor had infiltrated the body below the fin and a secondary melanoma was present on the dorsal edge of the tail, Text-fig. 8. Within a week after its amputation a blastema developed into an amorphous mass about 1 mm., Text-fig. 8b; within 17 days it was 2 mm., more vascularized, and contained four fin rays, Text-fig. 8c. Within one month the regenerating fin had six rays and a larger melanoma, Text-fig. 8d. At two months the new growth measured 4 mm.; it had destroyed all but three of the regenerated rays, Text-fig. 8e, and these were reduced to two within three months. During these three months the highly vascularized amelanotic melanoma reached its original size of 5.5 mm., Text-fig. 8f. The fish was then fixed for histological study.

The dorsal fin of another albino hybrid with an amelanotic melanoma measuring 1.5 mm. grew back after amputation to 4 mm., a size considerably larger than that of the original fin. The regrowth of the fin is thus not necessarily impeded by the simultaneous growth of a melanoma.



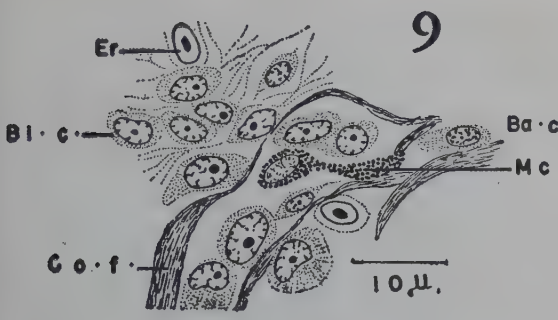
TEXT-FIG. 8. Regeneration of a large amelanotic melanoma in the dorsal fin of a platyfish-swordtail hybrid. 6 ×. **a**—Before amputation. **b**—Within 1 week following amputation. **c**—Within 17 days. **d**—Within 1 month. **e**—Within 2 months. **f**—Within 3 months.

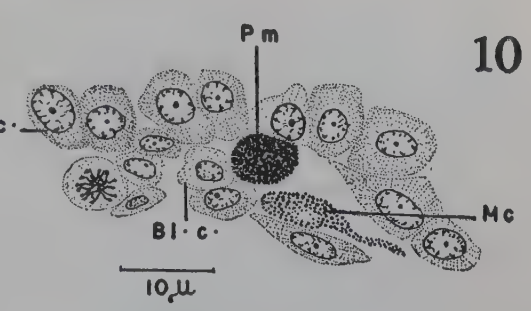
HISTOLOGICAL OBSERVATIONS OF REGENERATED DORSAL FINS

Histological observations of the regeneration process of the normal dorsal fins of platyfish and platyfish-swordtail hybrids having micromelanophores only (represented in Table 1, Nos. 2, 3 and 6) may serve as a basis for comparison with the regeneration process in fish having abnormally pigmented fins (represented in Table 1, Nos. 7, 8 and 9).

Within 12 hours after amputation of the normally pigmented fins, macrophages phagocytize melanin particles and other cell debris. The squamous cells of the epithelium moving from both sides of the wound grow over its surface to reform the epidermis. In the epidermis, the epithelial cells are fibrillar and loosely arranged; the macrophages that contain melanin particles are round or oval and have eccentric nuclei.

Within two or three days the basal cells of the adjacent epidermis move under the squamous epithelial cell layer. Collagenous fibres become more abundant in the connective tissues below the regenerating epidermis. Between these fibres are scattered fibroblasts, lymphocytes, granulocytes, macrophages and unengulfed melanin particles. The basal cells, some of which show mitotic figures, are 8 to 9 micra and their round or oval nuclei contain one or two nucleoli. The





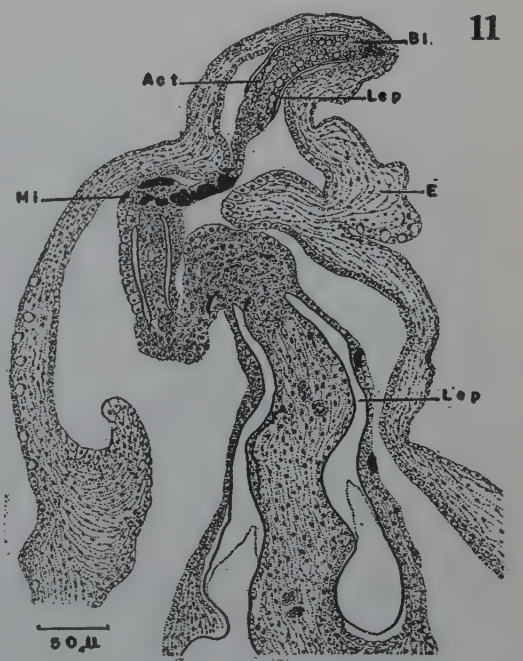
TEXT-FIG. 9. Details from blastema of regenerating dorsal fin of a comet platyfish, after one week, refer to Text-fig. 2b. **Mc**—Melanocyte between the blastema cells. **Bl.c**—Blastema cells. **Co.f**—Collagenous fibers at the base of blastema. **Er**—Erythrocyte.

TEXT-FIG. 10. Regenerating epidermis in the blastema, see Text-fig. 2d. Note one blastema cell in mitotic division. **Mc**—Melanocyte. **Ba.c**—Basal cells. **Bl.c**—Blastema cell. **Pm**—Pigment mass.

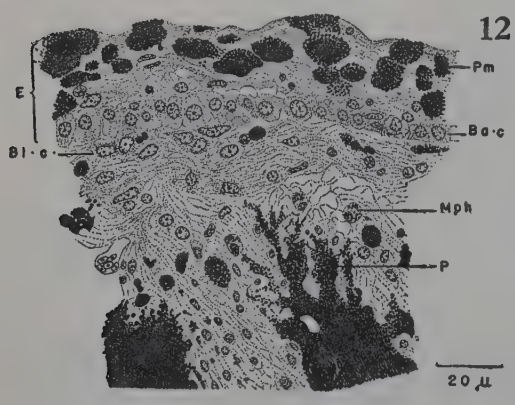
squamous cells are 5 to 7 micra and have elongated nuclei. The fibroblasts are round or oval, 5.5 to 7.5 micra, and have round or oval nuclei containing one or two nucleoli. The fibroblasts show some mitotic figures and are most active in the formation of the blastema proper, Plate I, Figs. 1, 2. The initial blastema contains no melanophores although its epidermis contains some free melanin and some macrophages with ingested pigment. Eventually the free melanin picked up by macrophages passes through the epidermis in the manner described by Bösenberg (1938) and by Gordon & Lansing (1943). Following this, melanocytes and young micromelanophores appear in the base of the blastema. The melanocytes are 14 to 15 micra; they are oval or spindle-shaped, but later they become dendritic. In Text-fig. 9 and Plate I, Fig. 3, a melanocyte is shown between blastema cells and collagenous fibres at the base of the regenerating fin, and in Text-fig. 10 another is shown just under the basal cells of the epidermis; here one blastema cell is in a stage of mitotic division. During the first week, blood capillaries appear in the blastema. Micromelanophores continue to increase as they move in along the connective tissue of the regenerating fin.

With regard to the regeneration of the fin rays, within one to two weeks after amputation, macrophages accumulate about the stumps of the fin rays and phagocytize the lepidotrichial debris. Later, the distal blastema cells begin to form actinotrichia and then lepidotrichia in a manner described by Blanc (1949). At first the regenerating lepidotrichia are not in contact with the ray stumps. Within three weeks those which form below the epidermis approach each other along their convex surfaces to reconstitute a fin ray. The proximal tips of the regenerated fin rays approach the distal tips of the old ray stumps and the distance between them is filled with additional skeletal elements from the blastema cells.

In Text-fig. 11, a diagonal cross-section of a dorsal fin 23 days after amputation, the lepidotrichia of two successive fin rays are shown. The epidermis of the fin is hyperplastic; the blastema



TEXT-FIG. 11. A diagonal section through a regenerated dorsal fin of a comet platyfish 23 days after amputation, see Text-fig. 2d. **Act**—Actinotrichia. **Bl** – Blastema. **E** – Epidermis. **Lep** – Lepidotrichia. **Mi**—Micromelanophore.



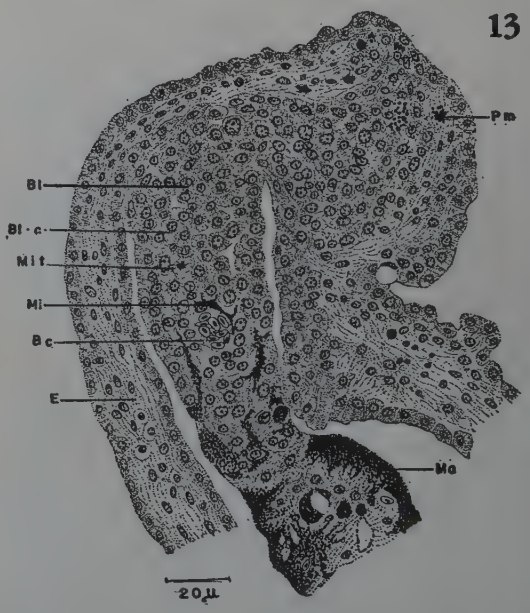
TEXT-FIG. 12. A section through a regenerating dorsal fin of a platyfish-swordtail hybrid four days following amputation of the fin, which had been in a state of melanosis, see Text-fig. 4c. **Ba.c**—Basal cell. **Bl.c**—Blastema cell. **E**—Epidermis. **Mph**—Macrophage. **P**—Processes of macromelanophore. **Pm**—Pigment mass in the epidermis.

cells are present in the distal part. The paired lepidotrichia of the old fin rays are located at the base of the fin. In this figure some micromelanophores are shown in the connective tissues around the fin rays. Within two to three months the regenerating fin reaches its previous dimension and then ceases to grow; the epidermal cells are no longer hyperplastic, the mucous and sensory cells reappear. Within four to five months the former pigmentation is restored in the regenerated fin.

From histological observations of the regeneration process, it appears that the most active growth takes place along the distal margin of the blastema, for it is there that mitotic figures are most frequently found.

Hybrids with melanosis of the dorsal fin reacted in a manner similar to the previous group following amputation, except for differences in pigment cell development. Within 12 to 48 hours following amputation of a melanotic dorsal fin, melanophore debris, free melanin, pigment-containing macrophages and collagenous fibres are present in the wound, Plate I, Fig. 4. Within three to four days the wound is covered by an epidermis and basal cells are restored. Macrophages and intercellular pigment masses are still present but they are eventually eliminated, Plate I, Fig. 5. The blastema cells accumulate under the basal cells of the epidermis, Text-fig. 12. This figure also shows some processes of macrophages that have entered the area of regeneration from below. Within six to seven days the blastema, which is covered by a hyperplastic epidermis, is formed, Text-fig. 13. Mucous cells in the epidermis are now present. Some blastema cells show mitotic divisions. Blood capillaries have developed. New micromelanophores are located in the blastema and macromelanophores are present in the base of the fin. Most of the macrophages and free pigment masses that were in the epidermis have been eliminated.

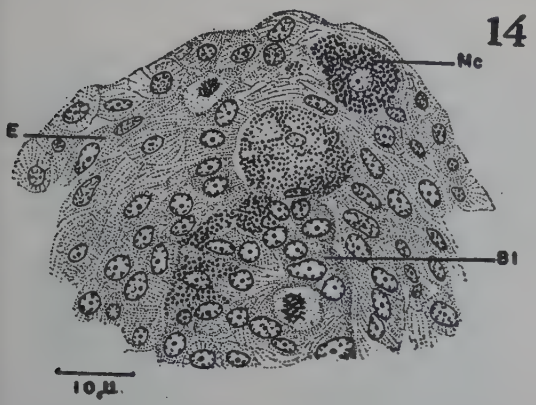
Melanocytes, the precursors of micro- and macromelanophores, are present between the blastema cells and in the epidermis, Text-figs. 14 and 15. They measure 11 to 22 micra and are round, oval cells, or spindle-shaped; later they may develop dendritic processes. It can not be said whether melanocytes come from the tissue under the epidermis or from the epidermis itself. Sometimes macromelanophores are located in the epidermis of the regenerated fin, Text-fig. 16. All of the macromelanophores apparently do not originate from melanocytes because macromelanophores that had been in the underlying tissues of the removed fin migrate into the regenerate, Text-figs. 4 and 17. As regeneration proceeds, the macromelanophores accumulate in



TEXT-FIG. 13. Cross-section of a blastema formed within six days in a similar fish, see Text-fig. 4. **Bc**—Blood capillary. **Bl**—Blastema. **Bl.c**—Blastema cell. **E**—Hyperplastic epidermis. **Ma**—Macromelanophore. **Mi**—Micromelanophore. **Mit**—Mitotic division in blastema cell. **Pm**—Pigment mass.

the connective tissue, where their pseudopodial processes anastomose and they recreate a state of melanosis in the regenerated fin, Text-fig. 18.

Two melanoblasts, 14 micra, each with fine melanin in granules, were found around a blood capillary of a one-month-old regenerated fin,

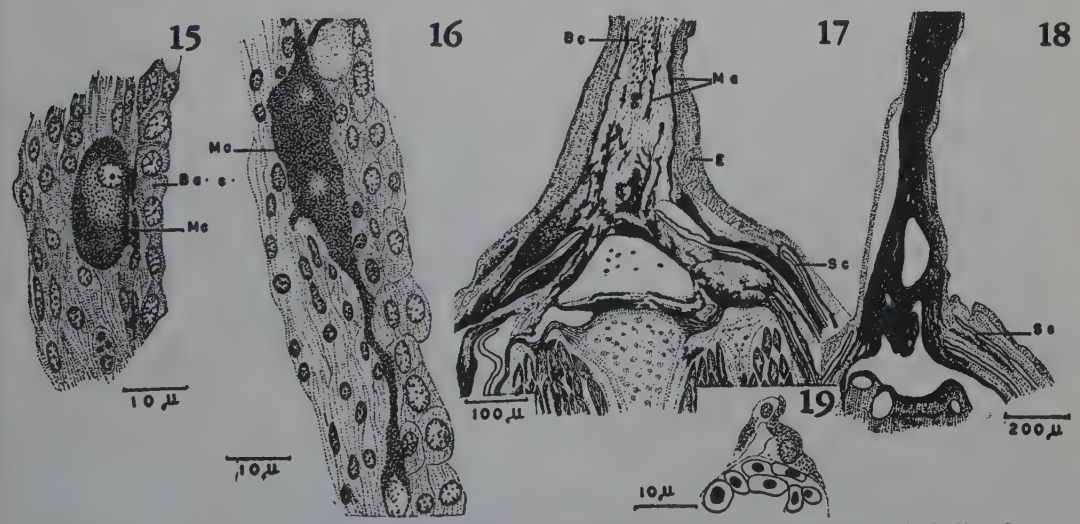


TEXT-FIG. 14. Details of melanocytes in the hyperplastic epidermis in the blastema of similar fin, see Text-fig. 4c. **Mc**—Melanocyte. **E**—Epidermal cell. **Bl**—Blastema cell.

Text-fig. 19. This suggests that new pigment cells in the blastema may develop *in situ*.

Fin rays may regenerate in the presence of melanosis, but in a few of these regenerating fins all the rays did not redevelop. Lepidotrichia first reform in the distal part of the fin, while pigment and epidermal cells generally reappear in the proximal region. Scales that have been destroyed at the base of the fin may also regenerate. Within two to three months the regenerated fin attains its former height, but the degree of its pigmentation is different. The developmental rate of melanosis in the regenerating fin depends upon the age of the animal and upon the degree of melanosis that exists on the body ventral to the amputated fin.

In hybrids that have melanotic melanomas in their dorsal fins, the regeneration process is similar, in the earlier stages, to that in hybrids whose dorsal fins show strong melanosis. Within 24 hours after amputation of the fin, the regenerating epidermis covers the wound surface, Text-fig. 20 and Plate I, Fig. 6. From these figures it may be seen that beneath the amputated fin, in the body proper, connective and muscle tissues had been replaced by tumor cells. The regenerating epidermis is hyperplastic and the cells are vacuolated. There are many macrophages and pigment masses. A bleached section of the fin is shown in Plate II, Fig. 1; the paired lepidotrichia appear darker here than in un-



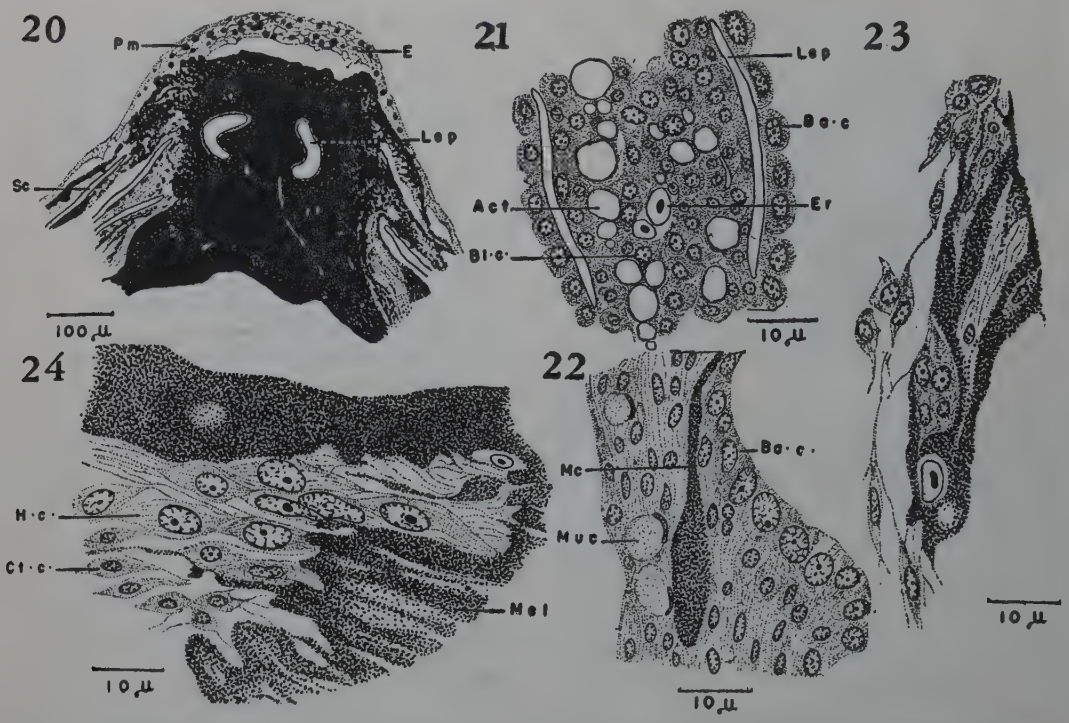
TEXT-FIG. 15. Details of melanocytes in the hyperplastic epidermis in the blastema of similar fin, see Text-fig. 4c. **Mc**—Melanocyte. **Ba.c**—Basal cells of the epidermis.

TEXT-FIG. 16. Macromelanophore in the regenerating epidermis of a blastema of a similar fish a month later, see Text-fig. 4f. **Ma**—Macromelanophore.

TEXT-FIG. 17. Cross-section of similar fish showing part of the regenerated dorsal fin within one month following amputation and part of dorsal region of body. Macromelanophores have migrated into the regenerated fin from the region of melanosis below dorsal fin, see Text-fig. 4f. **Bc**—Blood capillaries. **E**—Epidermis. **Ma**—Macromelanophore. **Sc**—Scale.

TEXT-FIG. 18. Cross-section through the regenerated dorsal fin after 6 months, see Text-figs. 4f, 4g. **Sc**—Scale.

TEXT-FIG. 19. Two melanoblasts around a blood vessel of a one-month-old regenerated dorsal fin, see Text-fig. 4f.



TEXT-FIG. 20. Cross-section through the regenerated epidermis of an amputated dorsal fin after 24 hours. The dorsal fin had a melanoma which extended into the region below the fin, see Text-figs. 6, 6a, 6b. **E**—Epidermis. **Lep**—Lepidotrichia. **Pm**—Pigment mass. **Sc**—Scale.

TEXT-FIG. 21. Distal region of a 13-day-old blastema of a melanomatous fish, see Text-figs. 5b, 5c. **Act**—Actinotrichia. **Lep**—Lepidotrichia. **Ba.c**—Basal cells. **Bl.c**—Blastema cells. **Er**—Erythrocyte.

TEXT-FIG. 22. Hyperplastic epidermis in a similar fish after 45 days, see Text-fig. 5e. **Mc**—Melanocyte. **Ba.c**—Basal cells. **Muc**—Mucous cell.

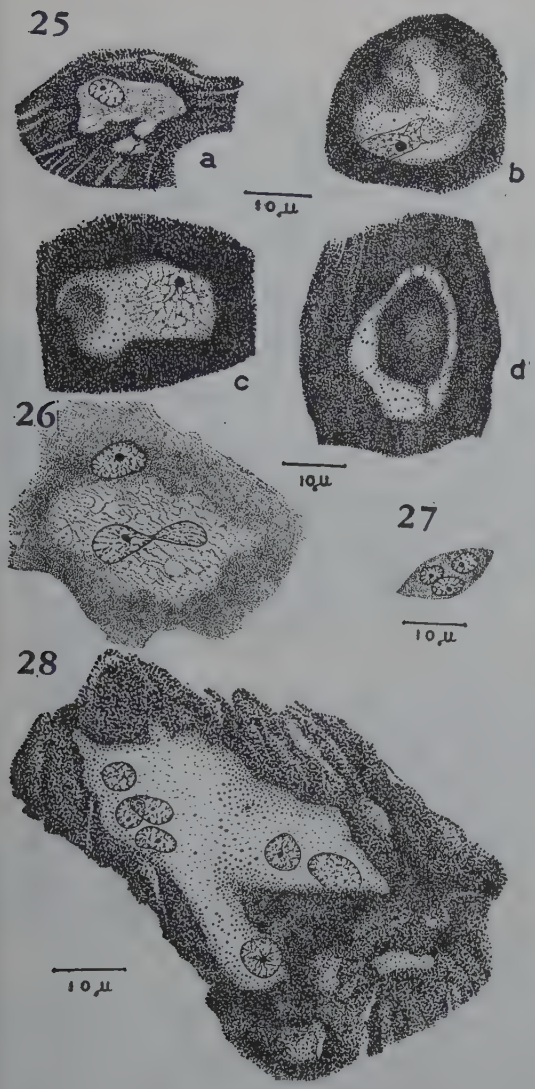
TEXT-FIG. 23. Macromelanophore surrounding a blood capillary in the connective tissue of a 13-day-old regenerated dorsal fin, see Text-fig. 5c.

TEXT-FIG. 24. Basal region of a regenerated dorsal fin, 13 days after amputation of a melanoma. The tumor cells' path of invasion of the regenerated fin follows the connective tissue, see also Text-fig. 6c. **H.c**—Hypertrophic fibroblasts. **Ct.c**—Fibroblasts, some are hypertrophic. **Mel**—Melanomatous tissue.

bleached sections, Plate I, Fig. 6. Within six to seven days the basal cells of the new epidermis appear over the wound's surface and the blastema is formed. The blastema cells, as is usual, originate from fibroblasts. In cases where the connective tissue beneath the amputated fin has been destroyed by tumor tissue, the blastema cells migrate from the nearest undestroyed connective tissues. Within 10 to 15 days actinotrichia appear, followed by the formation of paired lepidotricha, Text-fig. 21. During their regeneration some abnormalities occur, for example, sometimes three fin rays may regenerate to replace two.

Melanocytes and micromelanophores first appear in the blastema. Later, within two to three weeks, macromelanophores either migrate into the blastema from the region immediately below or they are reformed by melanocytes *in situ*. A melanocyte in the hyperplastic epidermis of a 2.5 months' old regenerated fin is shown in Text-fig. 22; Text-fig. 23 represents a macromelanophore around a capillary in the connective tissue.

After the condition of melanosis has returned in the regenerated fin, the hyperplastic growth of macromelanophores continues, the cells dividing amitotically. They become the principal tumor cells, replacing the connective tissue in the regenerated fin. The early fibroblasts become hypertrophic, Text-fig. 24; they measure 22 micra, are spindle-shaped, and their oval or round nuclei measure 8 to 10 micra. The extended pseudopodial processes of the macromelanophores first surround the fibroblasts, Text-



TEXT-FIG. 25. Pigmented hypertrophic fibroblasts at the base of a 13-day-old regenerated dorsal fin, see Text-figs. 6c and 24. **A**—Hypertrophic fibroblast surrounded by the pigmented processes of macromelanophores. **B**—Pigmented hypertrophic fibroblast, its melanin acquired from adjacent pigment-producing cells, macromelanophores. **C**—Pigmented hypertrophic fibroblast with nucleus in a state of karyolysis. **D**—Pigmented hypertrophic fibroblast with central mass of pigment granules.

TEXT-FIG. 26. Bleached section showing pigmented hypertrophic fibroblasts in amitotic division. The nucleus on the right is that of a macromelanophore which surrounds the secondary pigmented fibroblast, see also Text-fig. 6d.

TEXT-FIG. 27. Multinucleated spindle-shaped cell in the connective tissue of the regenerated dorsal fin, shown in Text-figs. 24 and 6c.

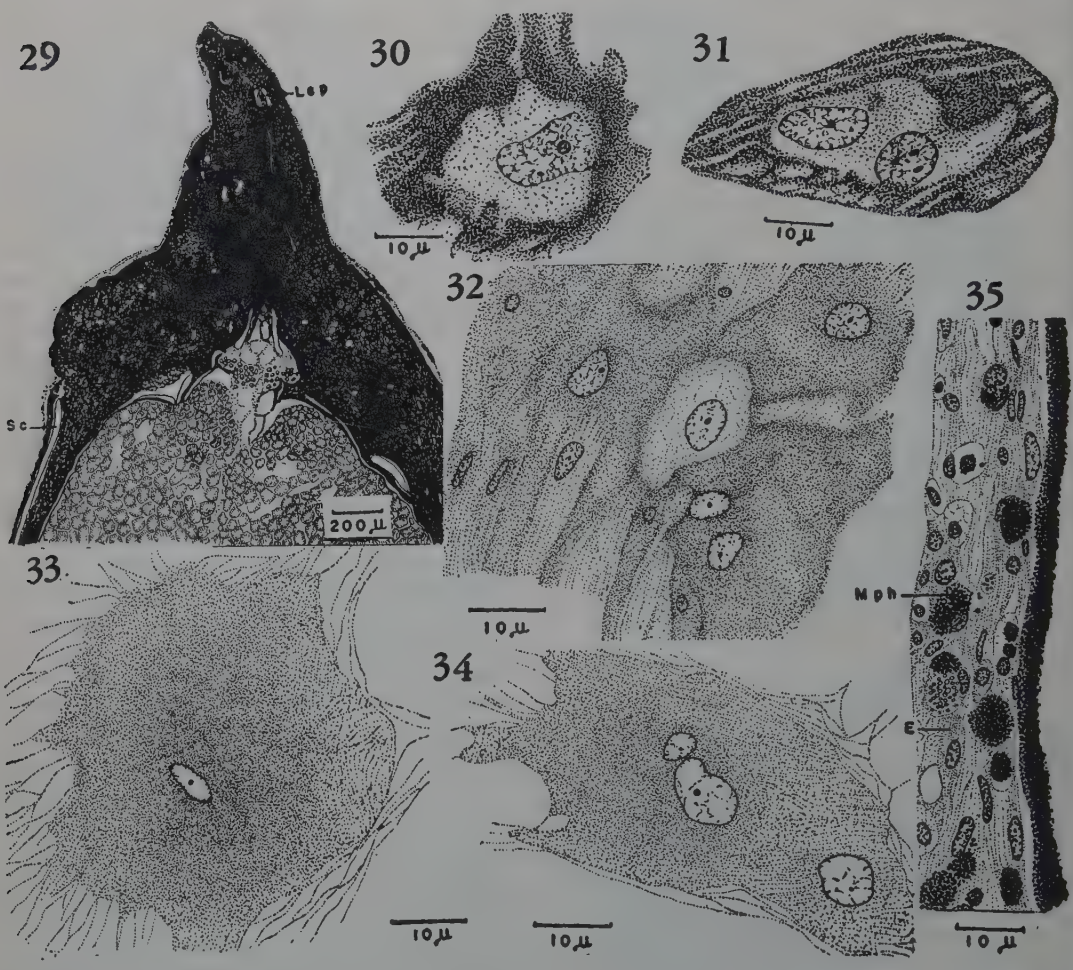
TEXT-FIG. 28. Giant cell with 7 nuclei from regenerated melanoma, see Text-fig. 6f.

fig. 25a. The fibroblasts obtain their melanin pigment by these contacts, Text-fig. 25b. The fibroblasts, which may be round, oval or polymorphic, increase to 25 to 35 micra and their nuclei measure 10 to 15 micra. Their nuclei divide amitotically with the result that in some cells two or more nuclei may be formed; this is illustrated in Text-fig. 26 which shows a bleached preparation. The nucleus at the periphery of the fibroblast shown in this figure also represents part of the macromelanophore which surrounds it. The fibroblasts which appear during the early stages of melanoma formation in the regenerate may be called "pigmented hypertrophic fibroblasts." The nuclei of some of these cells may disappear by the process of karyolysis, Text-fig. 25c. Melanin granules may reach the center of the fibroblasts and become concentrated there. Between this central mass of melanin in the pigmented hypertrophic fibroblasts and the surrounding macromelanophores, a nonpigmented ring may appear, Text-fig. 25d. The pigmented hypertrophic fibroblasts do not produce their contained melanin, but acquire it from adjacent pigment-producing cells. Some pigmented hypertrophic fibroblasts that developed in a melanoma are shown from a bleached section in Plate II, Fig. 2.

In the melanoma where pigmented hypertrophic fibroblasts develop, certain relatively small and polynucleate cells, measuring 18 to 19 micra, are occasionally found, Text-fig. 27. The cytoplasm of these rare cells stains more intensely with hemalum-eosin than the other cells in the regenerating tissues. These multinucleate cells possibly originate from the hypertrophic fibroblasts. In addition, some large polymorphous and multinucleate cells, which are about 500 micra in size and are called "giant cells," appear in the tumor tissue. One giant cell with seven nuclei is shown in Text-fig. 28. Probably these large cells are also derived from fibroblasts.

The connective tissue in the regenerated fin forms the fibrillar stroma of the developing melanoma. The connective tissue cells are relatively small, being 10 to 15 micra, and have nuclei 3 to 8 micra. Like fibroblasts, they may become pigmented during the hyperplastic growth of the macromelanophores. It is the hyperplasia of the tumor cells described above that causes a thickening of the fin which may even become nodular.

Macromelanophores are the principal tumor cells of the melanomas that redevelop in the regenerating dorsal fins. They measure 300 to 470 micra, their nuclei are 15 to 20 micra; they possess fine or lobulated dendritic processes. In the tumor tissue, they are found as individual cells or in complex aggregations forming syn-



TEXT-FIG. 29. Cross-section of regenerated melanoma that developed after 2.5 months in the dorsal fin in hybrid shown in Text-figs. 6, 6f. **Lep**—Lepidotrichia. **Sc**—Scale.

TEXT-FIG. 30. Pigmented hypertrophic fibroblast with one nucleus from melanoma shown in Text-fig. 29.

TEXT-FIG. 31. Pigmented hypertrophic fibroblast with two nuclei from melanoma shown in Text-fig. 29.

TEXT-FIG. 32. Bleached section of the dorsal fin melanoma shown in Text-figs. 6f and 29. Several macromelanophores form a syncytium within a fibrillar connective tissue stroma. A pigmented hypertrophic fibroblast is shown in the central area.

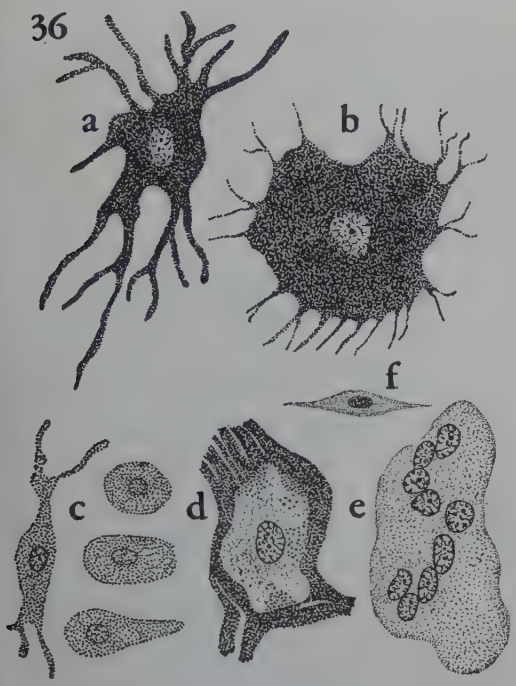
TEXT-FIG. 33. Bleached section showing an isolated macromelanophore from a melanomatous dorsal fin shown in Text-fig. 5f.

TEXT-FIG. 34. Bleached section showing an isolated macromelanophore with three nuclei from the melanoma shown in Text-fig. 5f.

TEXT-FIG. 35. Epidermis of a regenerated melanomatous dorsal fin, see Text-figs. 5e, 5f. **E**—Epidermis. **Mph**—Macrophage.

cytial masses in which cell boundaries can not be determined, Plate II, Figs. 3 and 4. In a bleached section of the melanoma, hypertrophic pigmented fibroblasts, connective tissue stroma cells and syncytial macromelanophores may be seen, Plate II, Fig. 5, The fibrillar structure of the cytoplasm of the macromelanophores may be seen clearly in this photomicrograph.

Two types of recurrent melanomas may be distinguished with regard to their origin and rapidity of growth. The fast-growing type is mainly composed of macromelanophores that have migrated into the reformed tumor from melanomatous tissues in the stump of the original dorsal fin and in the body just below, as shown in Text-figs. 6 and 29 and Plate II, Fig. 6.



TEXT-FIG. 36. Principal cells of the recurrent melanomas in the dorsal fins of platyfish-swordtail hybrids following amputation: **a** and **b**—Macromelanophores. **c**—Four melanocytes. **d**—Pigmented hyperplastic fibroblast. **e**—Giant cell. **f**—Stroma cell.

The tumor tissue in the regenerated fin, as well as in the basal region of the fin, have the same cellular elements and melanotic appearance. The processes of the macromelanophores lie parallel to each other or form swirls, Plate II, Fig. 4. Their cell boundaries are indeterminate, as indicated in the study of bleached sections. Most of these cells have migrated from their previous position in the basal parts of the removed fin and body, Plate III, Fig. 1. Other macromelanophores, arising from melanocytes, also participate in recreating the new tumor in the regenerated fin, but since they form a syncytial mass with the macromelanophores that migrate into the regenerating fin, it is difficult to distinguish between them. In this type of melanoma, pigmented hypertrophic fibroblasts, giant cells and connective tissue stroma cells are found. Two pigmented hypertrophic fibroblasts, one of which contains two nuclei, are shown in Text-figs. 30 and 31; Text-fig. 32, drawn from a bleached preparation, shows a pigmented hypertrophic fibroblast with several macromelanophores in a fibrillar stroma of connective tissue cells.

The more slowly-growing melanomas are those that require approximately ten months to redevelop *in situ*. These develop in hybrid fish exhibiting melanomas that were originally restricted to the dorsal fin, Text-fig. 5. They consist of isolated polymorphic macromelanophores which originate *in situ* from melanocytes in the regenerated fin, Plate III, Fig. 2. One hybrid developed a nodular melanoma in the posterior part of the regenerate, Text-fig. 5. Cross-sections of this tumor, Plate III, Figs. 2 and 3, show that it is not completely pigmented. The almost round or oval macromelanophores with fine processes are imbedded in a fibrillar and less pigmented connective tissue stroma. This may be seen in a partially bleached section of this tumor, Plate III, Fig. 4. Macromelanophores containing one or more nuclei are observed in other bleached sections, Text-figs. 33 and 34. The position of the lepidotrichia determines a radial arrangement of the fine processes of the macromelanophores in parts of the melanoma, as shown in Plate III, Figs. 4 and 5.

In both types of recurrent melanoma in the regenerated dorsal fins, certain tumor cells reveal a degenerative process in action, as indicated by pyknosis, karyorrhexis or karyolysis of their nuclei. Blood capillaries are well distributed throughout the melanoma and granulocytes and free erythrocytes are also present. The recurrent melanoma is usually covered by a thin epidermis, but in some instances the epidermal cells may be hyperplastic and may contain some macrophages and melanin masses, as shown in Text-fig. 35. The skeletal elements usually resist infiltration but they, too, may be destroyed by the tumor cells.

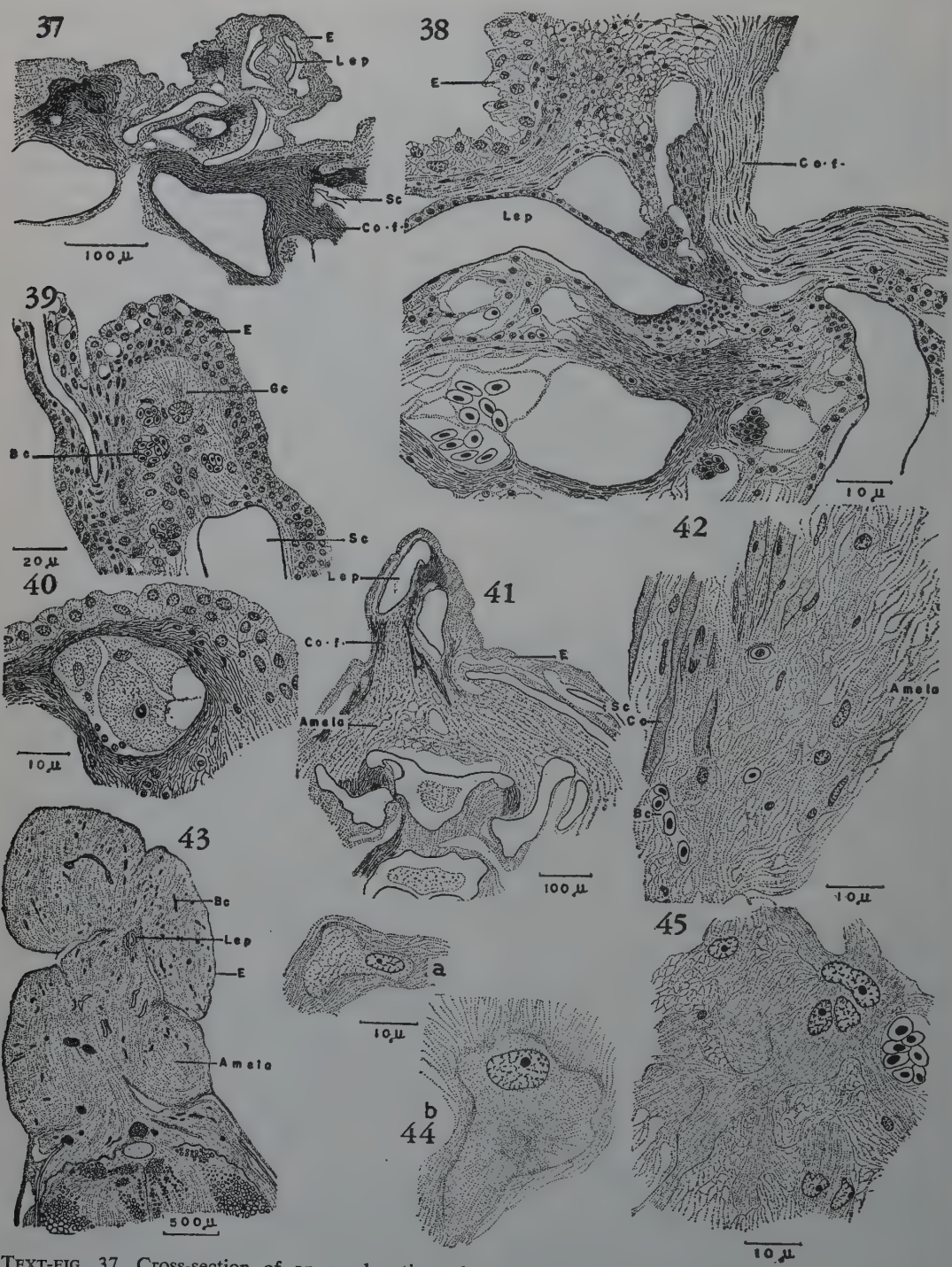
Thus, the first step in the recreation of melanoma in a regenerated dorsal fin is the development of a melanosis which is characterized by the proliferation and accumulation of macromelanophores. The macromelanophores then undergo progressive hyperplasia inducing, in turn, the hypertrophic growth of fibroblasts and giant cells. The macromelanophores invest these and other non-pigment-producing cells with melanin particles.

The most important pigmented cellular elements observed in the regenerating melanoma are as follows and are represented in Text-fig. 36:

a. *Melanocytes:* These are pigment-producing cells of 11 to 22 micra, with round or oval nuclei of 3 to 5 micra. They are usually oval or spindle-shaped, but may have wide lobulated processes. Melanocytes are capable of transforming into micro- and macromelanophores.

b. *Melanophores:* These are derived from melanocytes and are of two kinds:

Macromelanophores are large polymorphic cells measuring 300 to 470 micra, with round,



TEXT-FIG. 37. Cross-section of an amelanotic melanoma in the dorsal fin of a hybrid that reformed within 20 days after amputation, see Text-fig. 7c. **E**—Hyperplastic epidermis. **Lep**—Lepidotrichial element. **Co.f**—Collagenous fibers. **Sc**—Scale.

TEXT-FIG. 38. Section of the amelanotic melanoma shown in Text-fig. 37, under higher magnification.

TEXT-FIG. 39. Section of unamputated amelanotic melanoma tissue within a scale pocket at the base of the regenerated fin, see Text-fig. 7c. **E**—Epidermis. **Bc**—Blood capillary. **Gc**—Giant cells. **Sc**—Scale.

oval or polymorphic nuclei that are 15 to 20 micra; they contain one to two nucleoli and a loose network of chromatin. They have fine or lobulated dendritic processes. These are the cells that initiate the development of melanomas in platyfish-swordtail hybrids.

Micromelanophores are smaller, about 100 micra.

c. *Pigmented hypertrophic fibroblasts:* These cells have no dendritic processes. They are round, oval or polymorphic and measure 25 to 35 micra; their round or oval nuclei are 10 to 15 micra. They acquire their melanin from adjacent macromelanophores.

d. *Giant cells:* These are large polymorphic and multinucleated cells of about 500 micra. Their nuclei, which are round, oval or occasionally polymorphic, and are 5 to 10 micra in size, have a loose network of chromatin with one or two nucleoli. The giant cells may develop dendritic processes rarely; they also acquire pigment granules from true pigment cells.

e. *Pigmented connective tissue stroma cells:* These are spindle-shaped cells of 10 to 15 micra. Their oval or round nuclei measure 3 to 8 micra and contain dense granular chromatin. They, too, may acquire their small number of melanin particles from adjacent pigment-producing cells.

The regeneration process and recurrence of amelanotic melanoma in the dorsal fins of albino hybrids are essentially similar to these processes in dark hybrids with typical black melanomas. However, the cellular details may be more easily seen in sections of amelanotic melanomas and compared directly with bleached sections of ordinary melanoma.

The epidermis of a 20-day-old regenerated fin that had had an amelanotic melanoma is hyperplastic and many collagenous fibres are present, Text-figs. 37 and 38. The connective tissue and capillaries may be seen, as well as tumor cells, which remain below the amputated fin, in scale pockets and under the epidermis between the collagenous fibres, Text-figs. 39 and 40. The manner of infiltration by tumor cells into the connective tissue of a regenerated fin of the hybrid shown in Text-fig. 7 may be seen in Text-fig. 41. Some connective tissues and collagenous fibres which had formed in the regenerated fin are redestroyed and the fibroblasts are hypertrophic, Text-fig. 42. The nodular melanoma that developed in three months in the albino hybrid, shown in Text-fig. 8, when sectioned revealed the following details. The epidermis covering the overgrowth is almost normal but the vascularity of the amelanotic tumors is apparently greater than in the typical melanoma, Text-fig. 43 and Plate III, Fig. 6. Only three irregularly aligned dorsal fin rays are present in the tumor, the others being destroyed, Text-fig. 43. The connective tissue between the rays is replaced by the tumor cells, Plate III, Fig. 7. The trunk musculature at the base of the regenerated fin is separated from the melanoma by a layer of collagenous fibres, Text-fig. 43. In some areas this layer is destroyed by amelanotic melanoma cells which then infiltrate the musculature, Plate IV, Fig. 1.

The amelanotic melanomas in regenerated fins have a sarcomatous appearance histologically. The principal tumor cells are the amelanotic melanocytes and amelanotic macromelanophores. Some of these cells migrate into the regenerated fins from tumor cells below the fins, others are formed *in situ*. The colorless melanocytes and macromelanophores form syncytial masses in which cell boundaries are indeterminate. Their processes are sometimes parallel to each other; some form swirls, as

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TEXT-FIG. 40. Tumor cells surrounded by collagenous fibers at the base of a regenerated amelanotic melanoma, refer to Text-fig. 37.

TEXT-FIG. 41. Cross-section of a regenerated amelanotic melanoma in the dorsal fin after 3 months, shown in Text-fig. 7f. **Amela**—Amelanotic melanoma cells of the body invading the regenerating fin. **Co.f**—Collagenous fibers. **E**—Epidermis. **Lep**—Lepidotrichia. **Sc**—Scale.

TEXT-FIG. 42. Same as Text-fig. 41 but under higher power of magnification. **Amela**—Amelanotic melanoma cell from below the dorsal fin now in fin. **Co.f**—Collagenous fibers partly destroyed by tumor cells. **Bc**—Blood capillary.

TEXT-FIG. 43. Cross-section of amelanotic melanoma that developed in the dorsal fin of an albino hybrid within three months following amputation, see Text-fig. 8f. **Amela**—Amelanotic melanoma. **Bc**—Blood capillary. **E**—Epidermis. **Lep**—Lepidotrichial element.

TEXT-FIG. 44. Two types of tumor cells in amelanotic melanoma, refer to Text-fig. 8f. **a**—Cell with homogeneous cytoplasm, homologous to the pigmented hypertrophic fibroblast found in melanotic melanomas. **b**—Cell with radial fibrillar cytoplasm.

TEXT-FIG. 45. Giant cell with three nuclei in the amelanotic melanoma shown in Text-fig. 43.

shown in Plate III, Fig. 7, and Plate IV, Figs. 1 to 3. The cytoplasm of the macromelanophores has a fibrillar appearance. The melanocytes show mitotic figures, whereas the macromelanophores show amitotic ones. The cytoplasm of the non-pigmented hypertrophic fibroblasts in amelanotic melanomas is homogeneous and stains more lightly with hemalum-eosin than the other tumor cells, Text-fig. 44a. The non-pigmented giant cells have lobulated nuclei; some may be multinucleate, their nuclei dividing amitotically, Text-fig. 45, Plate IV, Fig. 4. Certain polymorphic cells (30 to 50 micra in size) in the amelanotic tumor have their counterparts in the pigmented hypertrophic fibroblasts of the typical melanoma. These polymorphic cells have fibrillar structures that radiate from a center (which can not be seen in the black melanoma), Text-fig. 44b and Plate IV, Fig. 5. Ermin (1946) described these pigmented hypertrophic fibroblasts as "sekundär" pigment cells which have one or more nuclei that measure 9 to 10 micra. Only the distal surface of the amelanotic melanoma was necrotic. Various types of cell degeneration, such as pyknosis and karyorrhexis, were observed. Sections of amelanotic melanoma, when compared with bleached sections of melanotic ones, reveal the fact that both types of tumor have almost the same fibrillar structure. Compare, for example, Plate II, Fig. 4, with Plate IV, Figs. 4 to 5. The lacunae described by Breider (1938) in gray melanomas on the bodies of "black albinos" and by Levine (1948) in amelanotic melanomas on the body proper—both of which are essentially the same—were not seen in the dorsal fin melanomas.

Finally, the amelanotic melanomas have some coarse granulocytes "in a discharging state" which are like those Catton (1951) described in various normal fishes and Aronowitz, Nigrelli & Gordon (1951) found in a spontaneous epithelioma in the platyfish *Xiphophorus variatus*.

Discussion

For studies of regeneration of the basic tissues of the dorsal fins and their pigmentation following amputation, certain fishes were chosen from many genetic stocks with a view of tracing the histories of two kinds of pigment cells, the micro- and macromelanophores. At first, fishes with micromelanophore patterns were studied; of these there were three kinds: (1) Wild type, *St* + +; (2) Wild type with a comet pattern, *St Co* +; (3) Wagtail, *St Co E*, see Table 1.

It should be noted that the micromelanophore pigmentation of the dorsal fin of the first two types is similar because the comet pattern is restricted to the caudal fin. In both dorsal and caudal fins of the wagtail, however, the number of these small pigment cells is much greater. Following amputation of the dorsal fins in all three types, the reformation of the fins and pigmentation requires about one month.

The sources of micromelanophores in the growing blastema and in the regenerated dorsal fin are: (1) from pre-existing melanophores on the base of the amputated fin and on the dorsal ridge of the body, just below the dorsal fin; and (2) from melanoblasts (or melanocytes) which come in with other cells to re-establish the dorsal fin, these pigment cells transforming *in situ* into melanophores.

This interpretation of the sources of melanophores in regenerated fins of fishes is essentially the same as those suggested by other observers, specially Bösenberg (1938), Goodrich & Nichols (1931), Wunder & Schimke (1935), Grimm (1949), Wunder (1951), Goodrich, Hine & Reynolds (1950), Goodrich & Bresinger (1953) and Goodrich, Mazullo & Bronson (1954), based on work on various species of freshwater and marine fishes. Bösenberg suggested that the melanophores were not migratory but were carried along with other cells into the regenerating fins. Goodrich, *et al* (1954), however, declared that some melanophores may enter as propigment cells and differentiate later; they are usually first observed as lightly pigmented cells having the migratory or ameboid form. Marcus & Gordon (1954) traced the movements of melanocytes in melanoma transplants and found that some melanocytes, after they transformed into melanophores, ceased moving.

The neural crest origin of pigment cells in lampreys and fishes was first suggested by Borcea (1909) and has been supported by Weidenreich (1912), Lopashov (1944), Newth (1951) and Orton (1953). But there may be other, as yet indefinite, sources of these cells, according to Oppenheimer (1950) and Goodrich (1950).

In the second series of experiments, the history of the restoration of the macromelanophore pattern in dorsal fins was studied in two genetic strains of platyfish: (4) Comet with spotted-dorsal, *St Co* + *Sd* and (5) Wagtail with spotted-dorsal, *St Co E Sd*. These were produced by intermating platyfish from two different geographical populations. The result in the next generation of this intermating, as shown by Gordon (1951a), is an increase in the intensity of macromelanophore pigmentation which reaches a point of atypical growth, that is, a low degree of melanosis. The spotted-dorsal fish with the comet and wagtail patterns were essentially similar in this respect. Somewhat similar also were the platyfish-swordtail

hybrids with various degrees of melanosis, item 7 of Table 1, *Sd*.

The restoration of pigmentation after amputation of the dorsal fin in *Sd* fishes depended primarily upon the state of original melanosis. For example, if the original melanosis was relatively light, then the regenerated fin was less pigmented. This confirms preliminary studies of this problem made by Goldsmith, Gordon & Nigrelli (1947). They found that the melanotic dorsal fins of five-month-old unoperated control platyfish-swordtail hybrids had more macromelanophores than the regenerated fins of 11-month-old sibling hybrids. They suggested that the difference might lie in the fact that the tissues comprising the regenerated fins are chronologically younger than those in the controls. In the present observations, if the dorsal fin originally had a strong melanosis, regeneration of pigmentation was more rapid and reached almost equal intensity after an 8½ month period. Younger hybrids had the capacity to develop the original state of melanosis more rapidly than older fish. This is interesting because Scott (1907) discovered that in *Fundulus heteroclitus* the regeneration rate of normal fins is greater in younger than in older fishes which, he believed, is in line with the theory that regeneration is a growth phenomenon.

In the reformation of the state of melanosis in the regenerated fins, one source of the pigmented cells, including the specific melanosis-producing macromelanophores, was through the migration of large melanophores from their position below the dorsal fin. Silber (1951) also found that macromelanophores entered the dorsal fin blastema of platyfish-swordtail hybrids from "pigment cell depots" located at the base of the fin. We have found a second source of macromelanophores in the regenerated fin, namely, that they are formed *in situ* from melanocytes. Recently Marcus & Gordon (1954) have also found that some melanocytes present in a transplanted melanoma transform into macromelanophores in host tissues.

After amputation, the reformation of a melanoma or an amelanotic melanoma in the dorsal fin of *Sd* hybrids (list in item 8, Table 1) depends upon the degree of tumor involvement not only of the original dorsal fin but of the body just below that fin. If the tumor development in the body below the fin is pronounced, the regenerated melanoma or amelanotic melanoma often exceeds the size of the amputated tumor, sometimes almost by three times. If there is little tumor involvement of the body below the fin, the reformed dorsal fin melanoma usually does not exceed that of the original tumor.

It is an interesting fact that the progressive growth of a melanoma may be halted temporarily by amputation of most of its tissues. Immediately after the operation, apparently there is sufficient normal tissue available in the stump of the dorsal fin to recreate the whole fin. Subsequently, the tumorous tissue that has not been removed grows, invades and destroys the newly regenerated tissues.

In the process of the reformation of the melanoma following amputation, the same sequences of tumor development were found as in the formation of the original melanoma. In hybrid fishes, the development of spontaneous and of regenerated melanomas is preceded by a premelanomatous state of melanosis in specific areas. In fishes carrying the sex-linked, dominant gene, *Sd*, the dorsal fin is the site of the melanosis, a condition brought about by the rapidly proliferating macromelanophores.

Gordon (1951a) pointed out that in pure platyfish, macromelanophores appear in the dorsal fin in response to the presence of the *Sd* gene only after three to five months, whereas in inter-racial hybrid platyfish with the *Sd* gene, these large pigment cells may appear in two weeks. In platyfish-swordtail, inter-specific hybrids, the macromelanophores in the *Sd* fish appear still earlier, some on the day of birth. Gordon (1948, 1950b) suggested that the rate of macromelanophore proliferation is accelerated in proportion to the strength and frequencies of other genes that modify pigment cell growth.

The atypical growth of these large pigment cells leads to a state of melanosis which may be destructive to adjacent normal tissues. The latter may be destroyed and replaced by them (Gordon & Smith, 1938). In the melanosis produced in platyfish-swordtail hybrids, the concentration of macromelanophores is so great that practically no other types of pigment cells can be distinguished. If the melanosis appears in the dorsal fin, as it does in *Sd* fishes, the fin may be destroyed at various levels, but there is no swelling of tissues. Gradually this phase changes and there appears a noticeable swelling. When sections are cut through the swollen areas and studied histologically, they reveal a significant change in cellular components. The outstanding feature of the new growth, as Reed & Gordon (1931), Gordon & Smith (1938) and Grand, Gordon & Cameron (1941) (by tissue cultures) have pointed out, is the preponderance of melanocytes and the relatively small number of macromelanophores.

The importance of understanding the transitional steps, from the appearance of macromelanophores in a genetically susceptible an-

imal, through their hyperplastic growth to the formation of a melanosis and, finally, to the development of a definitive melanoma, has been appreciated in former studies (Gordon, 1951b). But until the present work on regeneration and on transplantation (Marcus & Gordon, 1954), the cellular elements involved and their relationships to each other could not be properly evaluated.

There are two categories of pigment-carrying cells in the melanoma. One group contains those cells which not only carry melanin granules but are capable of synthesizing melanin pigment. This group includes melanocytes and melanophores, both large (macro.) and small (micro.). Pigment cells of the second group do not synthesize the few or many melanic granules that they carry. This group includes pigmented hypertrophic fibroblasts, pigmented giant cells, pigmented connective tissue stroma cells and pigmented macrophages (melanophages). These secondary pigment cells acquire their pigment by contact with cells of the first category through various processes.

In the transition from the state of melanosis to that of melanoma, the macromelanophores in their atypical growth form a dense syncytial mass in which their dendritic processes anastomose. Sometimes the melanophore processes are parallel, sometimes they form swirls. The cell membranes are indeterminate. The fibroblasts in contact with and in response to the progressive atypical growth of the macromelanophores, become hypertrophic and pigmented, possibly by cytocrine activity on the part of the dendritic, pigment-forming melanophores. Although we utilize Masson's (1948) concept of cytocrine activities, our use of the term *melanophore* is not the same as his; Gordon (1953) pointed out that many human pathologists have used the term *melanophore* to denominate what biologists call *macrophage*. The primary pigment cells, melanocytes and melanophores, have the property of liberating some of their melanin by clasmatosis (Grand, Gordon & Cameron, 1948). Melanin particles so released may be picked up by adjacent cells in the tumor, such as the fibroblasts, giant cells and other connective tissue cells. The pigmented hypertrophic fibroblasts may divide amitotically. These cells may lose their nuclei by the process of karyolysis. In some areas of the melanoma the fibroblasts form large oval bodies in which the acquired melanin is both peripheral and central. Similar pigmented bodies have been seen by Breider (1938, 1939, a, b), Ermin (1946) and Levine (1948). In another variation of the dorsal fin melanoma which externally appeared nodular, we have found macromelanophores of various configurations and with fine dendritic processes within a dense fibrillar connective tissue stroma.

The amelanotic melanomas, listed as 9 in Table 1, do not differ fundamentally from the typical melanomas except, of course, in the amount of melanin contained in the various cells. The details presented by Levine (1942) for amelanotic melanomas on the bodies of platyfish-swordtail hybrids have also been found to hold for those of the dorsal fin, except that the latter have a more prominant fibrillar network.

A comparison of the progressive stages in the regeneration of teleost fins with their normal development—based on the observations of many authors from Ryder (1885) and Harrison (1893, 1895) to Okado (1943) and Blanc (1949), and on the present studies—reveals that the regeneration process is essentially a repetition of the normal ontogenetic process. This similarity also holds for the restoration of the normal pigmentation patterns of the fins. Moreover, results obtained through the amputation of abnormal fins in a state of melanosis or with melanoma, show that here, too, the fundamental repetitive ontogenetic processes are evident.

It is well known that in the normal development of the teleost fin, temperature and other exogenous factors influence the nature of the growth process. Higher temperatures during certain critical development stages, for example, result generally in a smaller number of fin rays in the adult, and lower temperatures produce a higher count of rays, according to Hubbs (1922), Gabriel (1944), Täning (1952) and others. Recently Buser-Lahaye (1953) suggested that external influences (such as temperature and light) are not applied directly in the regeneration processes but are mediated through the endocrine glands among which the thyroid has a special role. In addition, although the part that nerve cells have been shown to play in regeneration in the amphibia has not been evaluated in fishes, it is probable that these cells are equally influential in teleosts. Indeed, the failure of some of the fin rays to regenerate in certain fishes may possibly be attributed to this factor.

Melanomas and other pigmented tumors in man and the normal pigmentation of the human body have been variously interpreted by pathologists with regard to cellular components and their embryological origin. This is evident by reading the more recent statements of Willis (1948), Masson (1951), Itô (1951), Becker (1948, 1953), Raven (1953) and Allen & Spitz (1953, 1954).

The subject is too involved for review here, but it seems to us that no discussion of pigment cells is complete without reference to Dawson's (1925) remarkable studies on human melanomas. We have found his well illustrated analysis of the progressive growth of human cutaneous melanomas most instructive, because he not only described the disease in its final, definitely pathological phase, but also he traced its ontological development from its earliest, apparently innocuous state. Studied by this dynamic method, the progressive history of human cutaneous melanoma shows striking parallels to those of fish. In comparing these histories it must be remembered that Dawson's term *melanophore* is equivalent to the biologists' *macrophage*. No true melanophores are found in mammals; melanophores are specialized effector cells characteristic of fishes, amphibians and reptiles. One of the important findings from studies of regeneration and transplantation of fish melanomas is that the true melanophore is related directly to the melanocyte and melanoblast. Thus it may be re-emphasized that the fundamental cell type in the melanomas of all vertebrate animals is the melanocyte.

Summary

1. The variously pigmented dorsal fins of fifty-three young and adult platyfish *(Xiphophorus maculatus)* and platyfish-swordtail hybrids *(X. maculatus-X. helleri)* were amputated. Their regenerated fins and pigmentary patterns were studied histologically.

2. It was possible to compare the results of amputation and regeneration of normally pigmented dorsal fins with those that were either in a state of melanosis or exhibited melanomas. Among the latter, it was also possible to compare the regeneration process in those that had typical black melanomas with those that had amelanotic melanomas.

3. All of the amputated dorsal fins regenerated in about two to three months, with essentially the same pigmentary pattern they showed originally. In some of the fishes with melanomas, regeneration was abnormal but it was not necessarily impeded by the simultaneous growth of a melanoma.

4. The restoration of melanosis in the regenerated dorsal fins was faster in young hybrids than in mature ones. The state of melanosis was incomplete in the regenerated fins of those fish in which the melanosis was confined to the fins alone. Melanosis was more complete in the regenerated fins of those fish that had melanosis in the fin and on the body below the dorsal fin as well.

5. The reformation of a melanoma in regenerated dorsal fins was more rapid in hybrids that originally showed a melanoma both in the dorsal fin and on the body ventral to the fin than in hybrids that had a melanoma in the fin alone.

6. The regeneration process and the recurrence of amelanotic melanomas in the amputated dorsal fins of albino hybrid fish were essentially similar to those in hybrids with typically black melanomas. The progressive growth of the melanomas was halted but only temporarily by amputation.

7. The same sequences were found in the reformation of the remissive melanomas that were observed in the development of the original melanomas.

8. After the dorsal fin was amputated, squamous cells of the epithelium moving from both sides of the wound grew over its surface and reformed the epidermis. Basal cells of the adjacent tissues moved under the squamous epithelial cell layer.

9. Melanocytes and micromelanophores appeared in the blastema before the macromelanophores. These are all true pigment cells that produce the melanin they carry.

10. Melanophores were derived from two sources: from normal pigmented areas immediately below the regenerating fin and from melanocytes that develop *in situ* in the blastema.

11. Some of the pigmented hypertrophic fibroblasts in the melanoma acquired their pigment from contact with macromelanophores either through the process of clasmatosis, cytocrine activity or both. This is also true of the pigmented connective tissue cells of the stroma and of the giant cells.

12. The regenerated amelanotic melanoma has a sarcomatous appearance histologically, as does the original tumor. The principal cells of the amelanotic melanoma are the amelanotic melanocytes and amelanotic macromelanophores.

Acknowledgements

We wish to thank James W. Atz for reading the manuscript, and Cafer Türkmen for aid in making the photomicrographs.

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EXPLANATION OF THE PLATES

Photomicrographs of sections stained either with hemalum-eosin or by Masson's method.

Plate I

Fig. 1. The regenerated epidermis five days following amputation of dorsal fin of a comet platyfish. The blastema cells are below the epidermis. (Corresponds to Text-fig. 2b, an earlier blastema stage). 240 ×.

Fig. 2. Bud formed of blastema cells pushes the regenerated epidermis upward. (Corresponds to Text-fig. 2b). 600 ×.

Fig. 3. A melanocyte (Mc) in a one-week-old regenerating dorsal fin. (Corresponds to Text-fig. 2b). 1000 ×.

Fig. 4. Epidermis four days following amputation of the dorsal fin that exhibited melanosis. The collagenous fibers below the epidermis and the epidermis cells surround free melanin masses. (Corresponds to Text-figs. 4b, c). 300 ×.

Fig. 5. The elimination of pigment particles through the epidermis of a nine-days-old regenerating dorsal fin of a platyfish-swordtail hybrid that had a melanosis. (Corresponds to Text-fig. 4d, at an earlier stage). 600 ×.

Fig. 6. The regenerating epidermis 24 hours following amputation of a dorsal fin of a hybrid that had a melanoma in the fin and in tissues below the fin. The remains of one fin ray may be seen within the melanoma tissue. (Corresponds to Text-fig. 6b at an earlier stage). 100 ×.

Plate II

Fig. 1. A bleached section through the regenerating dorsal fin shown in Pl. I, Fig. 6. 100 ×.

Fig. 2. Part of the melanoma in the ventral region of a regenerated dorsal fin of an *Sd* hybrid with melanoma. Numerous pigmented hypertrophic fibroblasts are present in the tumor. (Corresponds to Text-fig. 6e). 440 ×.

Fig. 3. Section showing parallel arrangement of dendritic processes of macromelanophores in the dorsal fin of an *Sd* hybrid with melanoma. (Corresponds to Text-fig. 6f). 440 ×.

Fig. 4. A bleached section of melanoma of the same specimen showing swirl-like arrangement of processes of macromelanophores. 440 ×.

Fig. 5. Pigmented hypertrophic fibroblasts and a syncytium of macromelanophore processes in a bleached section of a regenerated melanoma of an *Sd* hybrid that had a melanoma in its dorsal fin. (Corresponds to Text-fig. 6f). **Ma**—Macromelanophore. **P.h.f.** — Pigmented hypertrophic fibroblast. **S.c.**—Stroma cell. 1000 ×.

Fig. 6. Part of regenerated tumor after 2.5 months in an *Sd* hybrid that exhibited melanoma. (Corresponds to Text-fig. 6f). 600 ×.

Plate III

Fig. 1. Bleached section of the three-week regenerated dorsal fin of an *Sd* hybrid showing a macromelanophore that has migrated from the base of the fin. (Corresponds to Text-fig. 6d). 600 ×.

Fig. 2. Section of the nodular melanoma in the 10-month regenerated dorsal fin of an *Sd* hybrid showing macromelanophores developed in the tumor. (Corresponds to Text-fig. 5f). 600 ×.

Fig. 3. Nodular melanoma reformed in the posterior part of the same hybrid as shown in Fig. 2. (Corresponds to Text-fig. 5f). 50 ×.

Fig. 4. Same nodular melanoma shown in Fig. 3 under higher magnification showing radial arrangement of processes of macromelanophores. 100 ×.

Fig. 5. Part of a bleached section of the same nodular melanoma shown in Fig. 3. 600 ×.

FIG. 6. Section of amelanotic melanoma that had regenerated in the dorsal fin of an albino (*Sd i*) hybrid. Note the hyperplastic blood vessels. (Corresponds to Text-fig. 8f). 100 ×.

FIG. 7. Section of amelanotic melanoma of an albino hybrid showing the replacement of the connective tissue between the lepidotrichia by tumor cells. (Corresponds to Text-fig. 8f). 600 ×.

PLATE IV

FIG. 1. Section of amelanotic melanoma that redeveloped in the regenerating dorsal fin of an albino (*Sd i*) hybrid, showing the penetration and destruction of collagenous fibers at the base of the fin. (Corresponds to Text-fig. 8f). 600 ×.

FIG. 2. Section of an amelanotic melanoma showing amelanotic macromelanophores in the tumor tissue. (Corresponds to Text-fig. 8f). 950 ×.

FIG. 3. Another part of the amelanotic melanoma showing fibrillar structures in the tumor tissue. 600 ×.

FIG. 4. Two giant cells in an amelanotic melanoma. (Corresponds to Text-fig. 8f). 950 ×.

FIG. 5. Amelanotic melanoma showing cell (A) with radiating fibrillar structures. (Corresponds to Text-fig. 8f). 440 ×.

FIG. 1

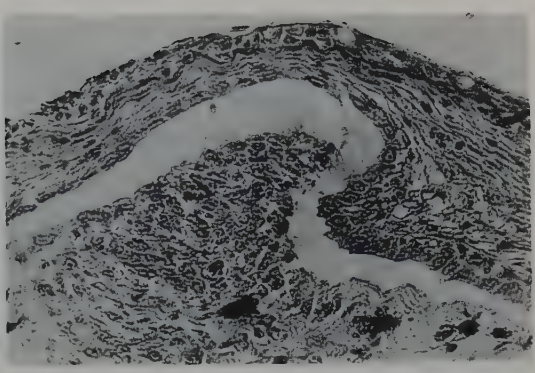

FIG. 2

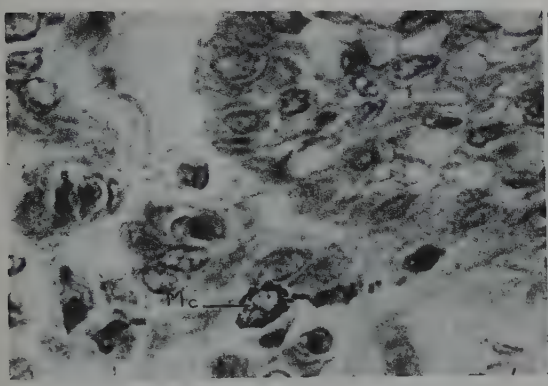

FIG. 3

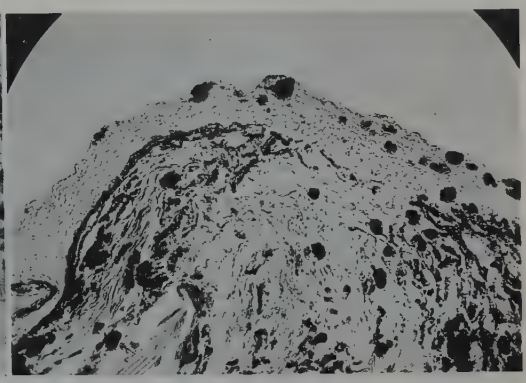

FIG. 4

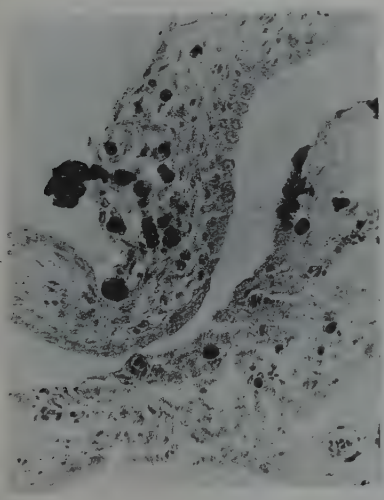

FIG. 5

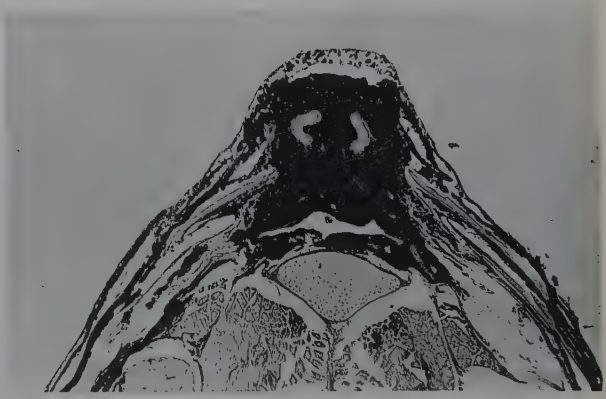

FIG. 6

REGENERATION OF MELANOMAS IN FISHES

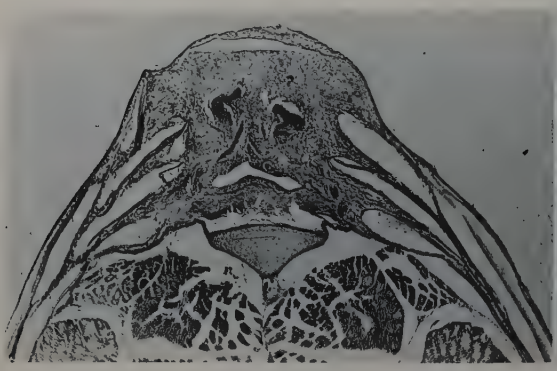

FIG. 1

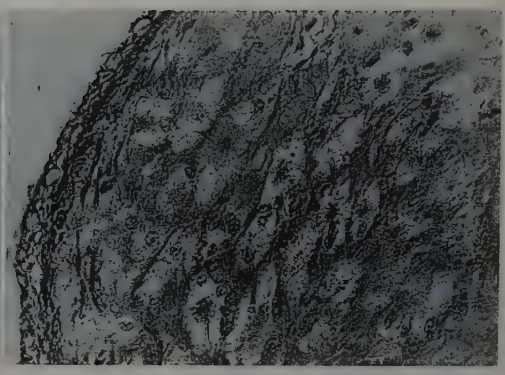

FIG. 2

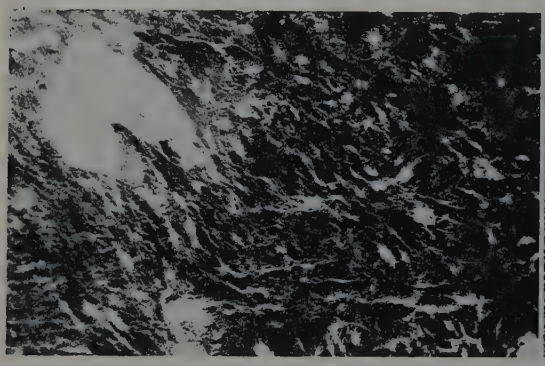

FIG. 3

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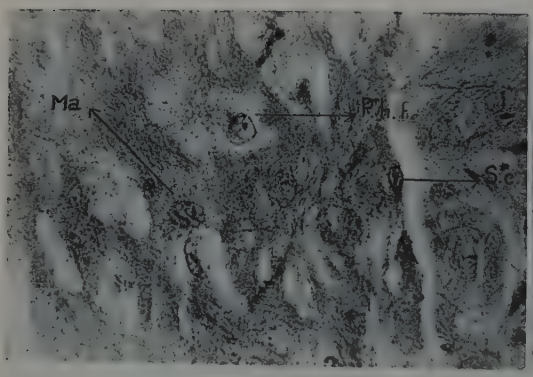

FIG. 5

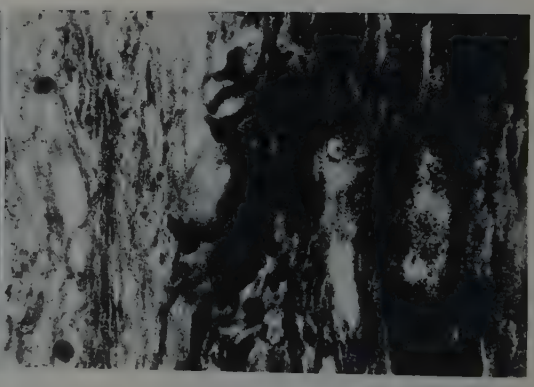

FIG. 6

REGENERATION OF MELANOMAS IN FISHES

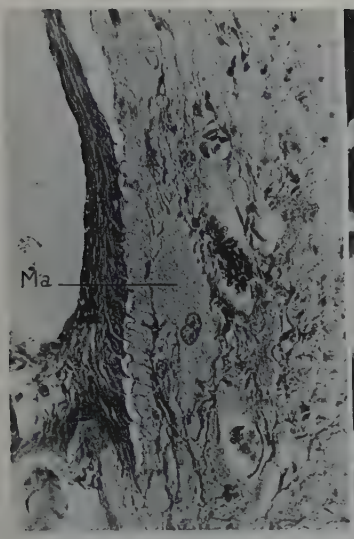

FIG. 1

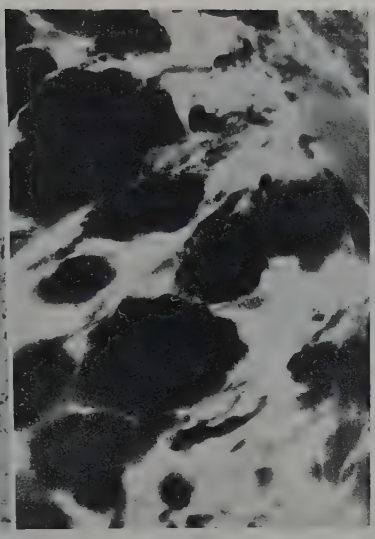

FIG. 2

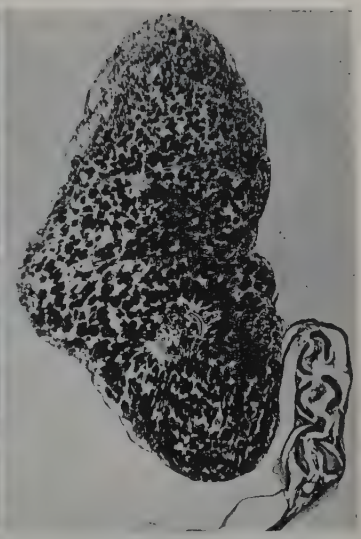

FIG. 3

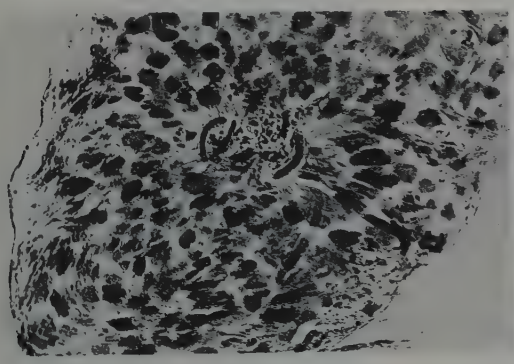

FIG. 4

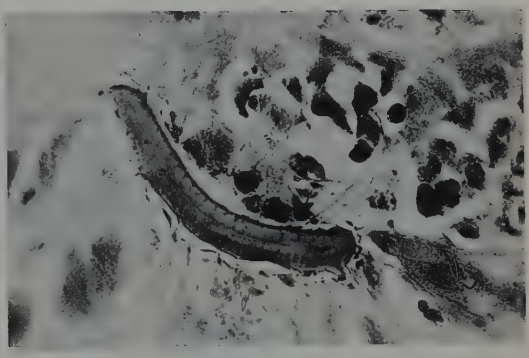

FIG. 5

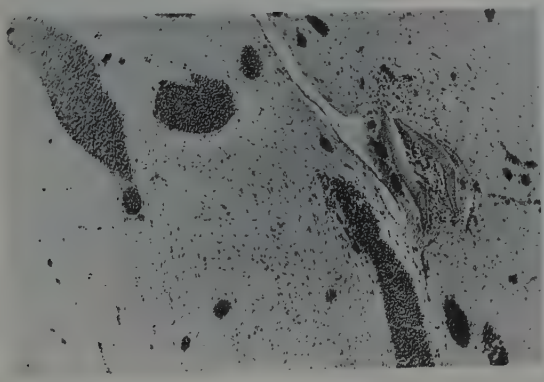

FIG. 6

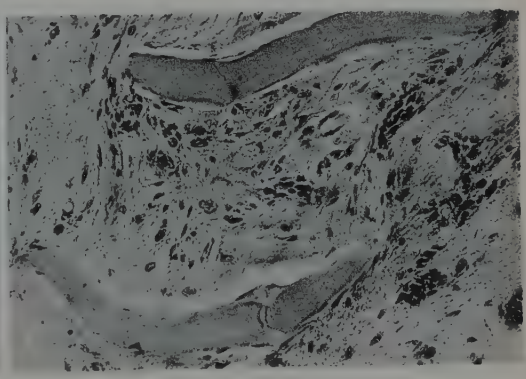

FIG. 7

REGENERATION OF MELANOMAS IN FISHES

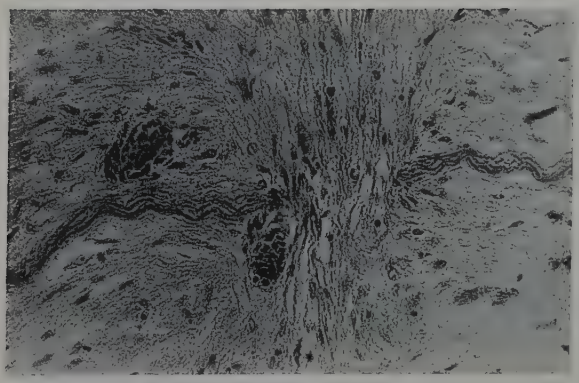

FIG. 1

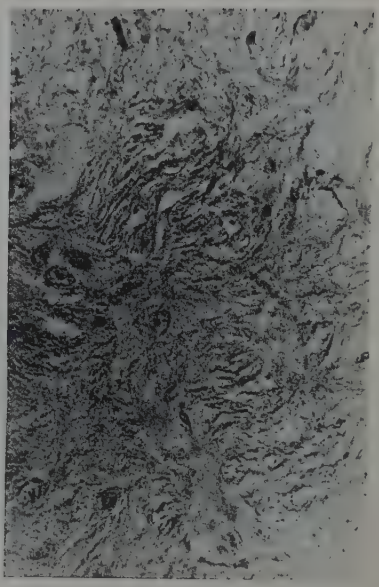

FIG. 2

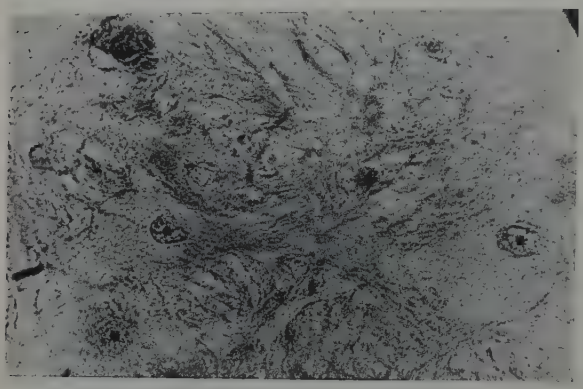

FIG. 4

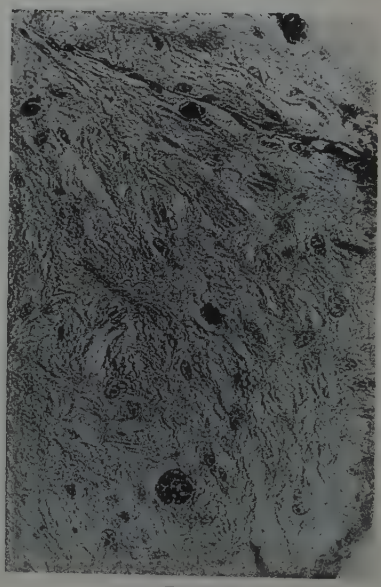

FIG. 3

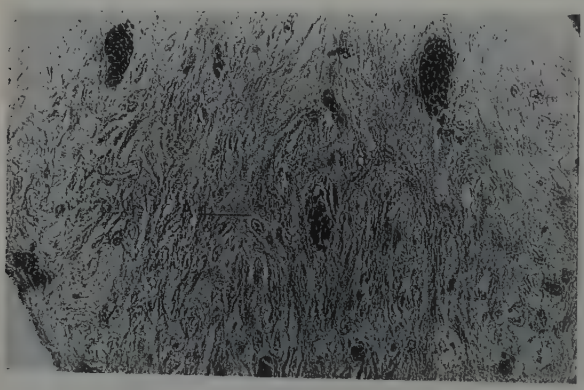

FIG. 5

REGENERATION OF MELANOMAS IN FISHES

7

Further Notes on the Pigmentary Behavior of *Chaetodipterus* in Reference to Background and Water Transparency

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(Plates I & II)

It has been shown by Breder (1946) that very small individuals of *Chaetodipterus faber* (Broussonet) appear in a coal black stage under certain conditions. When viewed against a light sand background on which similar-sized black gastropods and black infertile pods of the red mangrove were scattered, the small fish effectively vanished from sight. This black color phase was seen in fishes which were of about 10 mm. in length and which always inclined to one side. At larger sizes they were found to show the characteristic black and white vertical bars and upright position. The above observations were made on the west coast of Florida.

Subsequently, observations differing from these were made in the Bahamas, at the Lerner Marine Laboratory (Breder, 1948 and 1949). Larger-sized fishes, up to and including those a foot or more in length, were found there to appear not infrequently in a similar black phase. At such times they always lay on one side on the bright, light colored sand, appearing much like a piece of drifting trash, and could be easily overlooked. In Breder's 1949 paper it was suggested that the difference between the behavior of these fishes at the two places might be associated with the difference in the transparency of the water, that on the Florida west coast being notably turbid while the Bahamas water is remarkably transparent.

Various experiments were undertaken with some of these Bahaman fish in an effort to determine more clearly the basis of the differential behavior. In aquaria, under the most diverse conditions, they were found to swim upright and show at least some traces of the vertical bars, although the bars were generally less distinct when a minimum of dark objects was seen by the fish. This is, of course, in keeping with what had already been found.

Some of the experiments included presenting the fishes with variously painted backgrounds, such as broad dark vertical bars. In one series of experiments, quite accurate drawings of *Chaetodipterus* in groups in the solid black phase and in the barred phase were exhibited to a similar-sized test fish. None of these tests was able to make the fishes, solitary or in groups, obliterate the vertical bars, or recline as do the dark ones in the sea against a light sand background, or show especially vivid bars. It is to be noted that this is one of the species which does not show the classical concentration of melanophore granules in response to adrenalin (Breder & Rasquin, 1950).

A favorable situation led to the following clarification of some of the details of the characteristics which an environment must have to elicit the full expression of this dark coloration and reclining posture. A single individual about 3 inches long, in the dark phase, found reclining on its side near the laboratory dock, was transferred to a shallow circular concrete pool 12 feet in diameter. Here, over a bottom of clean light sand, the fish continued to perform as it had in the sea. Plate I, Figure 1, shows its appearance at this time. Both in this pool and earlier in the sea it permitted a very close approach and sometimes netting by the observer, simply lying very quiescent, but in about half the approaches it would dart away a short distance when very closely and persistently pursued. In this pool it normally took a position somewhere near the center and huddled toward the light blue walls of the construction.

After being transferred to a small aquarium, $2 \times 1 \times 1$ feet, with a similar white sand bottom,

the fish retained both its position and coloration as is shown in Plate I, Figure 2. These it held even when presented with various targets of black stripes and the figures of banded fishes which had been used earlier in the tests on other fishes. It did, however, show light bands but not very strongly, seemingly as a "fright" reaction in response to such things as taps on the glass walls of the tank.

On the coming of night and consequent lowering of light intensity the fish always swam erect and showed its bands. Such a condition is shown in the photograph in Plate I, Figure 3, taken by flashlight. Thus this behavior is evidently associated with light intensity, "fright" and a variety of visual stimuli, the latter perhaps being the least potent of the three influences. The puzzling responses studied earlier, wherein the fishes showed bands under all manner of aquarium fittings, were thus evidently actually in response to both fright and lowered light intensity. In these former experiments the aquaria were wrapped with paper on at least three sides and sometimes four, to insure the fish seeing a minimum of distracting objects, thus rather drastically reducing the light intensity.

Inasmuch as light intensity is evidently involved, and the turbidity of the water on the west coast of Florida reduces underwater illumination as compared with the Bahaman situation, the following experiment was undertaken. A suspension was made of pulverized charcoal and this was poured into the aquarium in broad daylight when the fish was in a dark, side-resting condition as shown in Plate I, Figure 2. The suspension made the water turbid and the larger particles settled out, transforming the aquarium bottom from one of nearly white sand to a mostly dead black condition. Immediately the fish erected itself and displayed the light, nearly white vertical bars, as is shown in Plate I, Figure 4, where the fish can be only faintly seen because of the turbid water. The following day the water had cleared but the bottom was largely black and the fish retained its stripes in bright daylight but over this dark bottom. This is shown in Plate I, Figure 5. Light sand was then introduced into the aquarium gently, through a small pipe, in order to bury the layer of charcoal. As this was late in the day, an artificial light was arranged so as to eliminate the effect of the decreasing light intensity. The fish returned to its black coloration and retained it long after daylight had faded. This is shown in Plate I, Figure 6. When this special light was extinguished, the fish then showed its bands as it had on other nights.

The following day the fish showed its daytime black color appropriate to a light sand background. Netting caused bars to reappear. It was then returned to the circular pool where it immediately resumed its dark phase and acted as it had before the aquarium experiments were undertaken. Late this day a small dark piece of Sargassum weed was dropped into the pool and by the following morning it had drifted to the outlet pipe. The fish was found under it and beside the black outlet pipe, vertical and in the strong black and white banded phase. When the fish was chased away from this shelter, it immediately obliterated the bands and reclined as a black object near the center of the pool on the light sand. It is to be especially noted that the outlet and inlet pipes in this pool were vertical and black (hard rubber) but that no attention had been paid to them by the fish until a sheltering, and shadow-casting, object was also associated with them. Similarly, Breder (1948) observed a large black fish, off the laboratory dock, which drifted slowly along the bottom on its side until it approached the pilings. It then became erect and showed its strongest bars as it swam among the rather thin piles supporting the dock. At that time it was thought that it was the sight of the dark vertical lines of the piles which elicited the response. In the light of the present series of experiments it would seem that this behavior is more probably referable to the shadow of the dock and the consequent lower light intensity, than to any definite retinal image.

The bold pattern referred to in this discussion is not to be confused with minor lightenings of the light barred areas which flash faintly in an evanescent manner following all manner of stimuli, including minor "frights" or the sight of food. These lesser responses are evident if watched for closely, and probably have a significance analagous to the twitching of a fin which these, and in fact most fishes, show under similar situations. They seem to be nothing more than nervous "starts." This individual, it must be emphasized, was from the first a most tractable aquarium inmate. An hour after it had been placed in the aquarium the fish acted as though it had always been there. It would investigate a finger outside the glass and it fed freely from this time on. When the light sand was introduced in the course of the experiments, the fish butted and bit at the pipe. It was clearly not nearly so timid as the fishes examined previously, which are referred to earlier in these notes.

The above experiments were performed on this single individual in November, and the fish was maintained in the laboratory until May when it was reintroduced to the circular pool with a light sand background. It responded in

a manner strictly comparable to its former behavior and differed only in that the blackening was not quite so complete and the reclined position not so nearly horizontal. This may be associated with the greater age of the fish, as there is considerable evidence that this behavior is most definite in the smaller sizes. On the other hand, it may be more truly associated with the long sojourn in aquaria. It is a commonplace among aquarists that many fishes after long residence in aquaria tend to show less vigor in their various responses than do wild fishes. Such a slackening of behavior vigor may be associated with waning health but certainly, in many cases, it is not so modified. In these instances it would seem to be more a matter of dropping old habits and developing new in accordance with the radical change in environment, brought about by moving from the open sea to a small aquarium, with consequent absence of predators and complete change in the manner in which food is presented or found.

Whether any or all of the above noted matters incident to captivity had anything to do with this slight change in pigmentary behavior is uncertain but they are mentioned here to indicate that the authors have not been unmindful of the possibility of such influences affecting the results. It is believed, moreover, that in the present instance the reduced light to which the fish was exposed in the laboratory aquarium for the period of some months was sufficient to induce a considerable reduction in the numbers of dermal melanophores. It may be noted that *Chaetodipterus* lives well, and for years, in public aquaria but little by little becomes much lighter. This lightening evidently results in part at least from the elimination of melanophores in the comparatively low light intensities of such places.

Because of the "opposite" pigmentary behavior as compared with that of usual background-matching species, the influence of melanophore-affecting hormones and other substances was investigated, as has been noted in passing by Breder & Rasquin (1950). The precise nature of these experiments is given below.

One specimen which weighed 276 grams and measured 192 mm. in standard length was injected with 2.8 cc. adrenalin 1:1,000. Throughout the observation period of four hours the injected fish remained noticeably darker than the uninjected control. Two minutes after injection, the injected fish was darker over the dorsal surface than the control; after six minutes it was still darker than the control but showed small white patches in the light bars. Ten minutes after injection the fish was darker than the control from the mid-dorsum down to approximately the lateral mid-line. It remained definitely gray where the control showed bold white bars. The black bars of the injected fish seemed less definitive than those of the control, although this may have been owing to less contrast of color offered by the injected fish. Two hours after injection the iris of the injected fish was white and the whole animal was quite dark, although not sufficient to eliminate the barred pattern entirely. After three hours the coloration of the iris had returned to normal while a mottled appearance was still evident on the body. After four hours the coloration of the injected fish was nearly back to normal, that is, it was nearly like that of the control. The following morning the injected fish and the control were indistinguishable.

Some observations were made on the pigmentary reaction to different backgrounds of three small individuals in a 15-gallon tank. No adrenalin injections were made. In a tank devoid of any plants or shells, with a bottom of white sand and with clear glass sides, the three fish assumed an all-over dark coloration with the lighter bands showing faintly. With a white sand bottom and with the sides of the tank covered with white paper, the coloration remained very dark with faint lighter bands. The fish appeared somewhat disturbed by this environment; they huddled together and were inclined to lean over to one side (Plate II, Figure 1). They were seen in the same dark color phase at night when the lights were suddenly flashed on in the laboratory.

In a tank with the slate bottom uncovered and the glass sides covered with black paper, the fish became lighter and the bands were more clearly marked (Plate II, Figure 2). They kept this banded condition when surrounded by black but quickly darkened when one paper side was removed for observation. All the fish swam upright in the dark tank and were not seen to lean over to one side as they did in the white tank.

With the sides of the tank covered with two-inch vertical black and white stripes, the fish assumed their bold black and white pattern. This reaction was not a quick one, but took about a half hour to occur. The fish again darkened quickly when one paper side was removed.

Paper images of the fish of approximately the same size were introduced into the experiment. They comprised white images on a black background, black images on a white background and white-barred images on a black background. The pigmentary reactions of the fish were the same when they were surrounded by any of these as backgrounds. They responded with the bold banded phase, even when black images on

a white background were used (Plate II, Figure 3).

One of these three fishes never gave as positive reactions as did the other two; it always remained somewhat darker and was seen more often in an inclined position than either of the others. It is possible that this was also an "emotional" reaction of some sort, especially as a dark phase is generally typical of most teleosts in the lowest position in a hierarchy, for this particular fish was annoyed and pecked at by the other two.

This type of pigmentary behavior, of which the present authors are evidently the only ones to take cognizance, at first glance might seem at wide variance with many of the pigmentary studies of recent years which have been broadly summarized by Sumner (1939), Walls (1942), Parker (1948) and Fox (1953). It is believed that the described observations on behavior can be entirely explained on the basis of experiments already performed on other fishes by various investigators. A considerable portion of it can be ascribed to the now well-established fact that tested fishes of various species show pigmentary responses both to light intensity and to the ratio of incident light to that reflected from the background. Walls (1942) treated the matter as follows: "If the fish were responding merely to the amount of light entering the eye, it should give the same responses to a brightly illuminated dark background as to a dimly illuminated white one—which would not adapt the fish at all! Instead, however, the shade assumed by the skin of the fish is always (unless the intensity of the incident light is very low or extremely high) in accordance with the albedo of the substrate—the percentage of incident light which the substrate reflects." Brown (1936) nicely demonstrated that the minnow *Erycimba* clearly responded to both variation in light intensity over a single background and to variations in the ratio of incident to reflected light, by using uniform light intensity and backgrounds of various degrees of reflectivity. Both his series of experiments were carried to the limit of the ability of the melanophores to respond; that is, to disperse or concentrate their melanin granules. Carried beyond, there was no further change. That is to say, below 0.000053 foot candles they showed no further concentration, as such was evidently impossible, while on the same black background, above 1.75 foot candles there was no further dispersion. The dispersion of the melanin was found to be proportional to the log of the foot candles at intermediate light values. Using the minimum light value necessary to produce complete dispersion on a black background, Brown varied the latter through various shades of gray to white; a light gray background which reflected 0.1411 foot candle of the incident 1.75 foot candles (an albedo of 12.4) was sufficient to produce full concentration of the melanin granules. The behavior at the other end of the series could not be tested because of the nature of the experimental arrangement whereby the minimum light intensity necessary to produce complete dispersion on black was employed.

The above-described experiments are sufficient to explain why dark or completely black fishes may show a pattern when the light has been reduced to a point where the presence of such a pattern cannot be seen. This would seem to have nothing to do with whether the fish tends to match the background or to contrast with it, but suffices to explain Figure 3 of Plate I. The phenomenon of a fully black fish on a light ground is apparently confined to places subject to very great light intensities. These often run up to and in excess of 6,000 foot candles in summer and usually well over 2,000 in winter at Bimini. Also the albedo is much smaller in such places than in most other natural environments, reaching values at least as small as 3.00. There is thus much less contrast between background and incident light than in places with darker background and less transparent water. That is, the sensible differential is much reduced, and the retinal polarizing effect is minimized. As noted, at these times the fishes recline so that one eye looks skyward and the other down, rather effectively neutralizing the polarization of the retina as compared with the condition when the fish is in the usual vertical position. Each eye, while seeing a different field, one differing from the other by the difference between the incident light and the reflected light, is nevertheless seeing a comparatively uniform field of very considerable brightness. This in itself may make it impossible for the fish to respond clearly to the albedo, responding instead overwhelmingly to the great light intensity, resulting in its darkening irrespective of the fact that the background is very light. This is the equivalent of saying that the polarizing effect of the visual field is essential in order for a fish *not* to respond only to the light intensity. Evidently only in regions of extremely clear water with an exceedingly light bottom is the described phenomenon possible.

The fact that these fish show their pattern in turbid water would follow from the above as turbidity would increase the value of the albedo because the incident light, whatever its value, only passes from the surface to the eye of the fish, while that of the reflected light has the much greater path; from the surface to the

bottom and back to the eye of the fish. Only in perfectly transparent water is the consequent reduction in light negligible. This is because the longer passage of the reflected light in even very slightly turbid water results in much greater filtering. Another type of behavior of *Chaetodipterus* which is encountered at Bimini and other places with similarly clear water, which has not yet been noted, is associated with deep water. In such places schools of five hundred or more individuals of medium to very large size may sometimes be seen, usually resting quietly and all headed into the current. Such schools have not been seen in depths of less than about six fathoms and the fish were always in a strongly barred phase. At such depths the growth-incrusted bottom does not reflect nearly as much light as the sandy shoal waters and consequently the albedo is of appreciable size.

That such fishes as *Chaetodipterus* do not show a general concentration of their melanin granules in response to an injection of adrenalin may be interpreted as follows. After injection of adrenalin the actual dispersion of the melanin granules in the lighter bars, the only place in the body where such activity could be detected, can only mean that the locus of the differential behavior between the two types of fishes is not to be found so much in a differently performing endocrine system but rather in a differently reacting set of target organs. That is to say, the same hormone, adrenalin, induces concentration of melanin granules in the dermal melanophores of *Gambusia,* but its dispersion in equivalent melanophores in *Chaetodipterus.* It is also to be recalled that melanophores of the iris and the meninges in both cases respond by concentrating their granules, as shown by Breder & Rasquin (1950).

In addition to this difference in cellular response it is probable that there is also a more subtle difference in the responsiveness of these pigmentary effects to nervous control. It is obvious that there is here an indication of a lessening endocrine control and an increasing nervous control with the teleosts of the more advanced grades. All the forms known to the authors which show this type of reversal of reaction are acanthopterygians, while the most typical examples of the simple background-matching types are non-acanthopterygians. The fact that these fishes showed faint evidences of momentary lightish bands as a reaction to "fright" when in the black phase, and a darkening of the bands under similar stimulae when in the banded phase, is suggestive of strong nervous control. Such clearly nerve-controlled changes are not evident in the more slowly responding melanophores of most non-acanthopterygians. Actually fishes from more turbid regions, *Chaetodipterus* included, generally show all-over lighter phases. In this extremely clear water, however, these fishes which are so much exposed to strong light have no doubt built up their complement of melanophores to maximum. The concentration of melanin in the areas which remain dark even when the fishes show their light bars is so great that it is doubtful if any hormonal application could cause a visible change in a short time. Evidently a sufficient reduction in the number of melanophores, to permit the noting of hormonally induced changes, could be effected by keeping the fishes under low light intensities for the required time. Whether there is an inverse behavior shown by the guanine present, as Hitchings & Falco (1944) showed for other fishes, cannot be established by these considerations.

A significant side to the differences in chromatic behavior which these fishes show under different conditions of turbidity is the fact that on the west coast of Florida *Chaetodipterus* is known to the natives as "white angel." This name is not used in Bimini, but instead the species is called "chirivita."[1] This appelation, elsewhere used for the very dark colored *Pomacanthus,* has evidently become transferred to *Chaetodipterus* at this place, while *Pomacanthus* is called "black angel."

The intermedin used (Choay) was found to be inactive on the melanophores of many different species of acanthopterygians. It was found to modify the melanophores of the freshwater characin *Astyanax* and the marine cyprinodont *Gambusia* but not to the extent of full granule dispersion. The biological assay for this hormone is performed on the erythrophore system of *Phoxinus laevis* and all fishes on which it was used responded by dispersion of granules in the erythrophores and xanthrophores. Because *Chaetodipterus* did not respond to this preparation of intermedin, it is not to be inferred that the melanophores of this species are not under control of the intermediate lobe of the pituitary, although, as has been noted, the nervous system plays a more dominant role.

The melanophore - dispersing hormone of Armour was not available at the time the above experiments and observations were carried out.

We wish to express our thanks to Miss Carol Mosher for technical assistance in connection with the November experiments and to Mr. William Clarke for observations in May which we were unable to make personally.

[1] Pronounced by the natives as "cherry-wheat-ah," which is in close agreement with their general habits of pronunciation.

Summary

1. The melanophores of the meninges and iris of *Chaetodipterus faber* concentrate their melanin granules on the injection of adrenalin, but those in the dermis simultaneously disperse their granules.

2. This species responds both to changes in light intensity and to the ratio of incident to reflected light from the background or albedo.

3. These conditions are evidently responsible for the fish showing a black and white banded phase in moderate light intensity with a large albedo, as in turbid water against a dark or mottled background, and in very low light intensities.

4. Alternatively they also cause the fishes to show a solid black phase in intense light against a very light background in clear water where the value of the albedo is very small.

5. These conditions lead to the interesting situation under natural conditions of a banded fish becoming inconspicuous against a variety of mottled backgrounds and again inconspicuous against a very light background by becoming uniformly black and appearing as a bit of sea bottom litter.

6. Accompanying these chromatic changes are appropriate attitudes, in the banded phase the fish swimming upright in ordinary fish fashion but in the black phase reclining quietly on the bottom or drifting slowly close to it propelled only by the hyline dorsal and pectoral fins.

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EXPLANATION OF THE PLATES

Plate I. Pattern reactions of a single individual *Chaetodipterus*.

Fig. 1. Behavior in a circular pool with light sand background. Fish black and reclining, as it did in the ocean.

Fig. 2. Identical behavior in a small aquarium floored with light sand.

Fig. 3. Nocturnal pattern, taken by photo-flash.

Fig. 4. Pattern in daytime after water had been turbidified.

Fig. 5. Effect of darkened bottom and clear water.

Fig. 6. Effect of renewed light bottom at night with artificial light. Aquarium photographs by C. Mosher.

Plate II. Pattern reactions of *Chaetodipterus*.

Fig. 1. Darkening of three individuals with the aquarium surrounded by white paper and light sand bottom, with no dark objects in the visual field. In these three photographs the paper on one side of the aquarium was necessarily removed just before the photograph was taken. This did not visibly affect the first two but did lighten the last, which effect increased after photography.

Fig. 2. Pattern of three individuals in an aquarium surrounded by black paper and a black slate bottom. The light appearance of the bottom is a refractive effect.

Fig. 3. Similar pattern shown by a single individual to which are displayed two black paper cut out "fish" with the aquarium surrounded by white paper and with a light sand bottom.

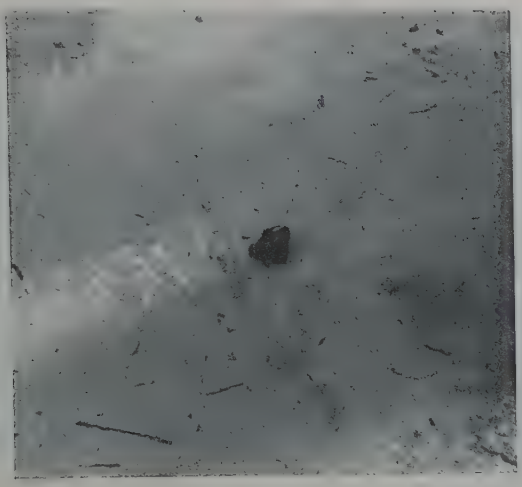

FIG. 1

FIG. 2

FIG. 3

FIG. 4

FIG. 5

FIG. 6

FURTHER NOTES ON THE PIGMENTARY BEHAVIOR OF CHAETODIPTERUS IN REFERENCE TO BACKGROUND AND WATER TRANSPARENCY

FIG. 1

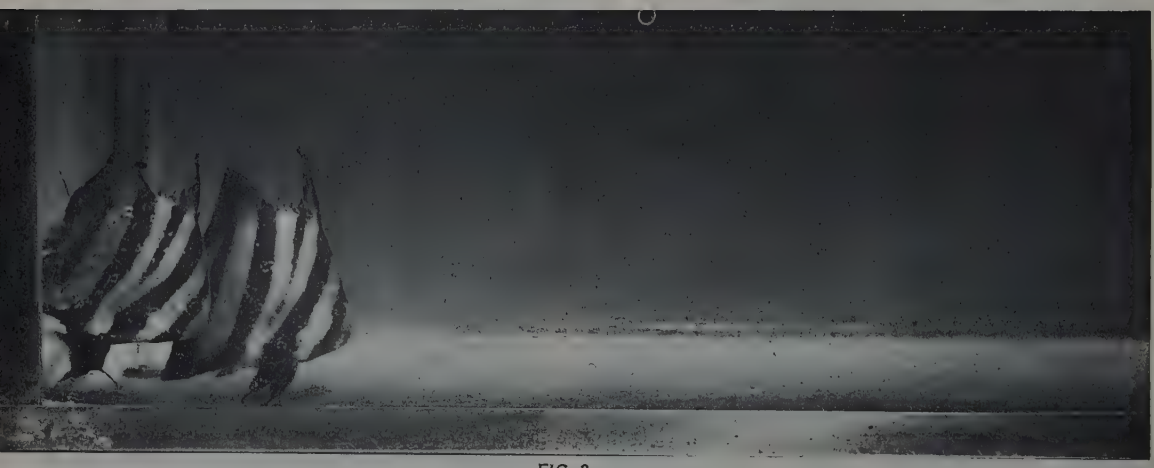

FIG. 2

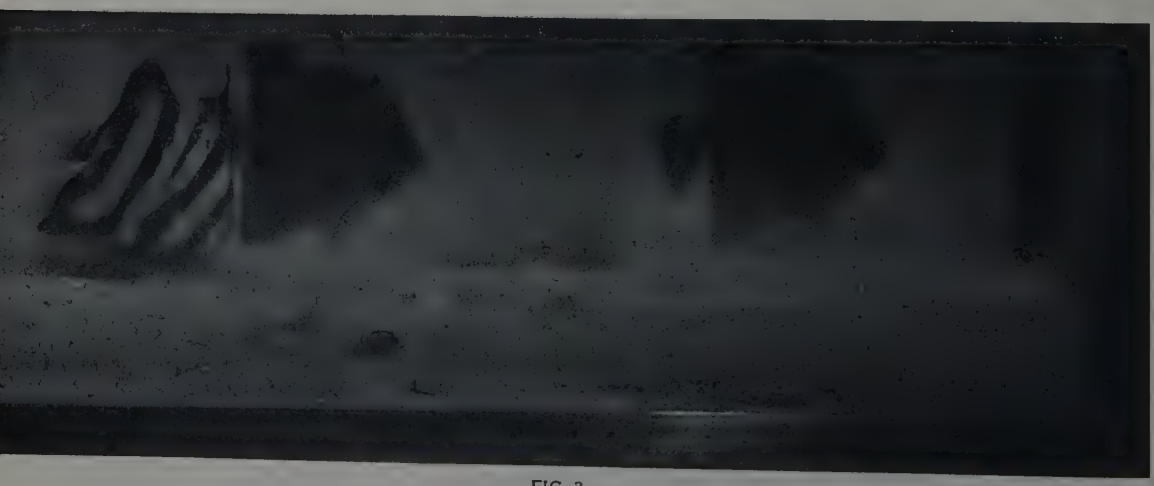

FIG. 3

FURTHER NOTES ON THE PIGMENTARY BEHAVIOR OF CHAETODIPTERUS
IN REFERENCE TO BACKGROUND AND WATER TRANSPARENCY

8

Special Features of Visibility Reduction in Flatfishes

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(Plates I-III)

Introduction

THE fishes of the order Heterosomata, the flatfishes, have many members which are remarkable for their ability to match closely the color of the background on which they rest and are evidently unique in their ability to match also the texture of the pattern of the background. Parker (1948), in noting this ability, wrote, "Responses to differences in pattern do not seem to have been observed in animals other than flatfishes; hence the exceptional nature of this group of teleosts." Mast (1916) published a series of plates in both black and white and color which clearly established the reality of this striking activity of the pigmentary system of flatfishes. Others, before and after him, who have contributed to our present understanding of background matching in this group of animals include Sumner (1910, 1911), Kuntz (1917), Schäfer (1921), Cunningham (1921), Hewer (1927, 1931), Meyer (1930, 1931) and Osborn (1939a, 1939b, 1939c, 1940, 1941a, 1941b).

These responses to background are exceedingly rapid and should not be confused with relatively permanent patterns which render a variety of other animals inconspicuous on some definite type of background. In the cases of the animals with a fixed pattern the disappearance is accomplished by the animal fitting itself against a suitable background and not by immediate and complex neural adjustments of the pigmentary system. A long list of forms which possess such comparatively stable patterns may be found in Cott (1940). These are widely distributed throughout the animal kingdom. Reports on various special cases of this type of behavior in fishes have been summarized by Atz (1951) and still others added by Uchida (1951). Obviously there is no sharp dividing line between animals which simply "melt" into the background and those which closely "mimic" some object, a division between such categories being often merely subjective.

There are, in addition to the problems of background matching, which have been studied by the workers mentioned above, other peculiarities in the pigmentary behavior of the Heterosomata. These, which include pigmentary behavior of the young and larval stages, have evidently never been studied nor related to the pigmentary behavior of the adults. Attention is herewith called to certain of these peculiarities and to some of the problems they pose in the Bothidae and the Achiridae.

Observations on Pigmentation and Behavior

The following data are based on original observations on pigmentary and other behavior of three species of the family Achiridae and one of the family Bothidae.

Patterns and Pigment in Achirids

Small individuals of *Trinectes lineatus* (Linnaeus)[1] of about 20 mm. in standard length are occasionally to be found near the laboratory and, so far, always in a solid black condition. One such individual, taken on January 15, 1952, was kept under continuous observation for more than five months in an aquarium provided with running sea water. This aquarium was floored with the typical light sand of the region, against which the fish was notably conspicuous. The fish at no time hid in corners of

[1] There has been considerable confusion in the nomenclature of these fishes. According to Chabanaud (1941), this form should be known as *Trinectes lineatus browni* (Günther), which see for a discussion of the matter.

the aquarium but regularly stationed itself on some centrally located portion of the aquarium bottom. Here it would lie, exposed on the very light sand, very often with no sand cover at all and seldom with more than a few grains. It was never seen to cover itself completely, a performance otherwise common in flatfishes. Some act disturbing the fish, such as pushing it about with a light rod, would not cause an alteration of this general behavior. It would usually move off slowly when the rod was thrust at it, with a peculiar form of locomotion resembling that of a large broad flatworm. It would so flute its marginal fins as to appear ruffled. Evidently the mechanics of this locomotion, which has been discussed in detail by Chabanaud (1941), is actually close to that of similarly-shaped flatworms. Once when so disturbed it shook off what few grains of sand were resting on it and rushed to nearly the exact center of the aquarium bottom, where it lay fully exposed and very conspicuous.

Lest it be thought that this was merely a melanistic individual which was unable to change its coloration at all, it may be pointed out that after dark the fish, discovered by flashlight, would always be found to be exhibiting the more usual cross bands (see Plate I, Figures 1 and 2).

Since most blinded fish in strong light develop their darkest phase, on which subject Parker (1948) gives a brief historic review, the presence of vision in this individual was carefully checked. This could be readily established by the accuracy of its striking at such things as *Artemia* as well as by the associated eye and body movements.

The above notes and photographs were made during January. By May the fish had altered its pigmentary behavior to the following. During the daytime it alternated between burying itself in the sand, so that it could only be seen partially or not at all, and exposing itself. At these latter times it came to show its former night time pattern with increasing frequency during the daylight periods. The coal-black phase appeared less and less often and usually the fish was a slate gray with black bars or dark brown bars. Sometimes it would swim about holding little piles of sand on various places on its flat body. The aquarium in which all this change transpired was near, but not directly, under a skylight. The significance of the resultant reduction of light intensity, as compared with the open harbor, is considered later.

Another sole, *Trinectes inscriptus* (Gosse), with a fairly inconspicuous pattern and color, always buried itself completely and with extreme rapidity, in the same sand. This action was so fast and with such immediate vigor that it was impossible to photograph the fish against a sand background. The photograph of this fish, Plate I, Figure 3, which appears to be that of the fish resting on the sand, was actually taken when an expedient was devised to circumvent the fish's rapid response. The fish was placed in a glass bowl which contained water only and then the bowl was placed on top of a pile of sand. The violent action of the fish in its fruitless attempts to bury itself was "stopped" photographically by using an electric photoflash. Although this fish, as may be noted from the photograph, is not exactly conspicuous on the sand of the area it inhabits, it still does not nearly approach the better background matches so usual in these fishes. As far as could be determined from aquarium observations, this fish was completely nocturnal.

On the west coast of Florida, at the Palmetto Key Laboratory of the old New York Aquarium, in 1940, eggs of *Trinectes lineatus* collected by tow net were carried through metamorphosis in finger bowls. At the time when the one eye began its migration to the other side of the head, general pigmentation was well advanced so that the fish was fully pigmented before the transformation was completed. This fish, as the eye migrated and the pigmentation increased, behaved in a manner suggesting that this period of change in the relationships of the visual fields was one of considerable behavioral difficulty. At first it swam at an angle as though attempting to retain a horizontal axis between the eyes. After this there followed a period, as the eye came over the dorsal profile of the head, in which the fish would lie down briefly, then get up and swim about erratically and then lie down again. Finally these periods of reclining became longer and longer, until the fish began to behave in a typically flounder fashion, the wandering eye, meanwhile, attaining the other side of the head. The water at this place is very turbid because of the presence of an extremely rich plankton, a condition which, as will be developed, has a distinct bearing on these pigmentary matters. Pigmentation appears in this form before the egg is hatched and Plate I, Figure 4, shows its extent five days after hatching, but long before any evidence of transformation appeared. The developmental stages of this species have been figured by Hildebrand & Cable (1938)[2] and the present material is in complete agreement with their illustrations.

Transparent and Pigmented Bothids

Small planktonic and fully glass-like individ-

[2] The same species in the usage of Chabanaud (1941).

uals of *Platophrys ocellatus* (Agassiz) may be taken about submerged lights in Bimini harbor with some degree of regularity. They are in an advanced stage, with the eyes on one side, and generally are found floating passively just under the surface of the sea. When placed in a bowl or aquarium they promptly settle to the bottom. Four views of such an individual are given in Plate II, two by transmitted light and two against a dark background. Oblique light was used in all but the first in order to take maximum advantage of the structural interference with light. It will be noted that the eyes are fully pigmented, the photographs with the dark background showing that the color of the investing tissues produces a light eyeball. This is a near cream color and very close to that of the light sand of this region.

In this stage these fish are extremely interesting to watch through a low power binocular. Mostly they rest quietly and show much oculomotor activity. Their extremely mobile and, of course, independently moving eyes, are capable of looking straight up. It is almost startling to have one such eye appear suddenly to be looking back at one through the other end of the microscope. That this great ocular activity is utilitarian is indicated by the fact that nearly always some particle is clearly in the line of fixation. That this really is the case may be demonstrated by the introduction of a quantity of newly hatched *Artemia*. The near ones become the objects of fixation just a moment before a small darting movement and engulfment of these food objects takes place. Because of the fish's great transparency, the swallowing of the *Artemia* may be easily followed to where it quickly lodges in the stomach. As the stomach becomes packed with *Artemia* and they die and are acted on by digestive juices they become a pale "boiled shrimp" pink which beautifully outlines the stomach. As the stomach does its work, small boluses of this material are seen to tear away from the main mass as the pyloric sphincter opens to pass the food along into the intestine. The whole process of digestion may be followed in this manner as though viewed on the screen of a fluoroscope.

If these fish are permitted to live under such conditions but with the normal sand background they nearly completely disappear, only the pigmented eyes being recognizable but, even so, extremely inconspicuous. Plate III, Figure 1, shows such a situation. The eyes may be seen as indicated but the mottling about them consists of the sand grains seen through the body of the fish. When this photograph was taken a few pigment spots had appeared, but earlier even these were not to be found. The fish may continue to live in this condition for a protracted time, not changing in pigmentation as rapidly as do many other planktonic larval fishes when brought in contact with a shore-like environment in an aquarium. These two fishes were taken about a submerged light on November 22, 1953. The photographs of one of them in Plate II, as noted in the legend, were taken on Nov. 23, 24, 26 and 27 respectively. Figure 1 of Plate III, which includes the two fish, was taken on Dec. 12, twenty days after they had been living in a sand-floored finger bowl. Two days later the next photograph, Plate III, Figure 2, was taken with the condition practically unchanged. Finally on December 19, after 27 days in the bowl (Plate III, Figure 3) the larger individual was seen to be fully pigmented and the smaller nearly so. In both the transparent and pigmented states and in the intermediate conditions these fish seem to be just about equally invisible. At this time it was necessary to abandon this series of observations, but these examples were carried to a point where the essential pattern of the adult had established itself. It may be noted that in all the fishes shown in Plate III, there is a small dark median spot about two-thirds of the distance from the eyes to the caudal peduncle. This made its appearance between the time the first of these pictures was made and the last of Plate II.

Discussion

From the foregoing descriptions it is evident that all flatfish pigmentary behavior is not uniformly a matter of matching the color and texture of the background. The following tabulation indicates the situations involving pigmentary and other behavior with regard to various environmental conditions which have been herein described.

Pigmentary Condition	Location of Fish	Light
	Trinectes lineatus	
Uniformly black	Exposed	Intense
Barred	Exposed	None
Barred	Exposed Buried	Moderate
	Trinectes inscriptus	
Reticulated	Buried	Intense or Moderate
Reticulated	Exposed	None
	Platophrys ocellatus	
Transparent	Exposed	Moderate
Background matching	Exposed	Moderate

The normal habitat of all was one of light sand, extremely clear water and extremely bright sunlight, excepting that of the Floridian

Trinectes. These reactions, which at first might seem to be merely fortuitous and without any regularity, can in fact be readily interpreted and understood in the light of the known behavior of teleost pigmentary systems. The explanations covering the situation in each of the four species examined follow.

The case of the Bahaman *Trinectes,* black on a light sand background, is completely explained on the same basis as the similar behavior of *Chaetodipterus* discussed at length by Breder & Rasquin (1955). As they indicate, in a fish responding to both light intensity and albedo under conditions of intense light and very light background, the melanophores will show maximal dispersal and the fish become substantially black. In very low light or none, as at night, what basic pattern is present but hidden reappears. Therefore there is no necessity to attempt to ascribe some biological utility to the seemingly pointless matter of "displaying" a pattern when it cannot be seen. After this fish had been kept in a laboratory aquarium for a long time it evidently reduced its amount of melanin in response to the lesser light intensity and showed its pattern in the daytime.

Trinectes inscriptus, which showed no evident pigmentary responses, simply remained nocturnal, with a pattern which was neither strongly contrasting with the background nor closely matching it.

Trinectes lineatus, living in a place of relatively low light and high albedo, showed both good matching and burying habits and had well pigmented larvae.

The glass-like planktonic young *Platophrys ocellatus* may settle to the bottom in that condition and so remain for some time and then gradually develop the extremely accurate bottom matching behavior so characteristic of the group.

One of the most interesting matters that developed in connection with the contemplation of these fishes was that their behavior, in each case, was appropriate to their pigmentation. The transparent, the closely bottom matching and the strongly bottom contrasting fishes all exposed themselves and each, in its way, thereby became less visible. Only those which were neither extremely contrasting nor extremely well matched to the bottom, hid or buried themselves. Note especially that the Bahaman *Trinectes,* at first so extremely black and "bold," became barred after a sojourn of some months in the less intense light of the laboratory, and changed its behavior appropriately. These clear changes in behavior in accordance with pigmentary differences add emphasis to the observations that fishes may employ either an extremely well matching pigmentation or a violently contrasting one to disappear effectively. That there are other fishes which darken on a light background in a strong light and thereby become inconspicuous was recognized by Breder (1948, 1949) and by Breder & Rasquin (1950, 1955).

The *Trinectes* taken from turbid water with a comparatively dark bottom was kept in a light colored bowl in the laboratory. It regularly lightened at night and darkened in the daytime. Here it seemed to respond chiefly to light intensity in a manner comparable to that of clear water *Trinectes* but less vigorously under the lesser stimulus. This followed from the extremely shallow water in the light bowl being insufficiently turbid to produce an albedo of large value, such as was normal to the environment.

In the case of the transparent *Platophrys,* as a purely practical matter it would seem to make little difference whether these fish remained transparent so that the bottom could be seen through them or whether they developed a very complicated pigmentary system which resembles the bottom under them as much as possible. The reasons for this change from the transparent to the pigmented, both of which evidently have equivalent value to the possessor, is probably to be found elsewhere. There may be two chief reasons for this change. It is conceivable that it would be difficult to prevent the development of pigment in the bright environment in which they live. It may be impossible for any large fish to remain transparent in such places, which circumstance would in effect relegate this device to application by only small organisms and for periods of relatively short duration. It also may not be very practical behavior to display the physiology of digestion through transparent sides on a sufficiently large scale. This demonstrated digestive activity might very well be attractive to the numerous marauding crabs of the region. Aside from these possibilities, there may be purely physical obstacles to the maintenance of transparency for long in such an environment. That is, some of the radiation may be physically injurious to certain tissues if long continued. It is thought, at least, that the comparatively subdued light of the laboratory made it possible for these fishes to retain their transparency longer than they could have in the wild state. The fact that *Platophrys* showed no change in general behavior, in passing from its transparent to its closely matching pigmentary pattern, would seem to indicate that the fishes were equally "secure" at all times during the transition.

In a more general consideration of teleost pigmentary reactions, reference may be made

to some earlier studies of such matters. Breder (1949) found it convenient to prepare a table which listed the conceivably useful reactions a fish might find it possible to make in response to various stimuli. Pigmentary response was only one of the items listed and only that part of it which refers to reactions to background pertains to the present considerations. This fragment of the tabulation may be rephrased for present purposes as follows.

Possible pigmentary reactions in reference to background:

A. Matching background
   a. In general tone
   b. In pattern detail
B. Opposing background
C. Indifferent to background

The data presented here show the fishes to subscribe to the items in this tabulation as below.

Trinectes lineatus	B., A., a.
Trinectes inscriptus	C.
Platophrys ocellatus—Transparent	C.
—Pigmented	A., b.

It is thus evident that each of the categories listed is represented in various ways by these fishes. The transparent *Platophrys* is considered "indifferent to background" in that it does not respond, not having any chromatophores with which to respond. *Trinectes inscriptus,* in the same category, is pigmented but there are no prompt pigmentary reactions. Each of the categories, as represented by these fishes, is primarily appropriate for the "freezing" type of organism, except that shown by *Trinectes inscriptus,* as an example of "C." This is the only fish which showed violent burying attempts. It is to be noted in this connection that *Trinectes lineatus* showed pronounced burying attempts only after it passed from its extreme background contrasting condition.

As a matter of general observation involving such species as *Pseudopleuronectes americanus* (Walbaum) and *Paralichthys dentatus* (Linnaeus), both excellent bottom matchers, the following conditions seem to obtain. They may be found either exposed and bottom matching or partially or fully buried with only their eyes above the sand. If pursued in shallow water over a uniform bottom they are not likely to bury themselves unless the pursuit is very vigorous. However, if they are driven onto a differently colored or textured bottom they are almost certain to bury themselves. This is, of course, in keeping with the data herein presented. Evidently the burying behavior supplements the pigmentary behavior in accordance with the requirements of the moment. This inter-relation of locomotor and pigmentary response to background has been shown, in a different guise, to be present in such simple background-matching fishes as *Gambusia,* by Breder (1947), and in *Cyprinodon,* by Breder & Rasquin (1951). In both cases the activity of the fishes was found to greatly alter when they passed over a non-matching background. Also related to this is the reluctance of many fishes to move over a background toned differently from the one on which they have been swimming for some time, a matter discussed in the references above.

Some of the features associated with the development of glass-like transparency, common to the planktonic young of many fishes, have evidently not been previously discussed. Since various details of the present data suggest some of the necessary concomitants of a practically invisible body, they are discussed below.

It is noteworthy that in all the innumerable fishes that have such transparent early stages the eye is well pigmented. This is, of course, essential to image formation on the retina, and hence to vision. Evidently not one known fish has developed transparent but sightless eyes. Such a development would clearly extend the transparency to practical perfection. From the fact that this has not been done, it is deduced that vision plays such an important role in the lives of these fishes as to forbid its abrogation in the interest of perfection of transparency as a protective mechanism. There would thus seem to be a balance between the two conditions, in which the value of the retention of vision overrides the significance of cues which a predator might find from the presence of two "disembodied" black spots moving along a fixed distance apart. As an item of interest bearing on the above, very often, on examining a bucket or bowl of plankton, the first knowledge that the viewer has of the presence of the more extremely transparent fishes is just this indication of paired black dots moving along together without any apparent reason. This is, of course, a sophisticated recognition which the uninitiate do not usually make. If predators on these fishes are able to use similar means of detection it is evidently not sufficiently important to detract seriously from the survival value of combined transparency and vision.

Considerations of the relationship between the transparent fishes and the opaque ones follow. It would appear that certain limited tissues, such as muscle, cartilage and connective tissue, may be rendered completely transparent while others, by their very nature, cannot. The latter type includes at least erythrocytes with their contained haemoglobin. In the transparent fishes

such unavoidably colored tissues are evidently kept at a minimum. Rasquin (in press), for example, in discussing the leptocephalus of *Albula*, shows that the red blood cells are remarkably few. With such structures kept at the minimum and dispersed in the animal, its visual density at macroscopic levels may be no greater than that of the surrounding water. To attain this condition the opaque tissues would have to be dispersed in the body of the animal in a manner approximately equivalent to the dispersion of particles suspended in the water and of their order of magnitude.

In a similar sense, pigmented fishes, both background matching and background contrasting, may disappear by mingling with particles of detritus which are of their own order of corporal magnitude. As found in a state of nature in water, most bodies either float or sink. Because of this, such association with particles about the size of the fishes in question is practically absent from mid-water but such hiding at the surface or on the bottom is common and widespread.

The next step in such a series by a single fish is the matching of a large number of smaller particles or objects. This evidently is done by the animals which closely resemble an area of background with which they are associated. This frees them from the need of having something sufficiently like themselves in size with which to mingle. There is no better illustration than a flounder matching a pebble background.

These observations lead to a general consideration of fishes living in extremely clear and brightly lighted water where optical cues are probably dominant or at least have much greater range and importance than in turbid water. That the interplay of the three most prominent methods of reducing conspicuousness, i.e., transparency, background matching and background contrasting, is most evident in brightly lighted clear water, should be expected. Likewise it should be expected that these features would be relatively reduced in turbid waters. The turbidity itself sharply restricts the range of vision while the increase in albedo which it induces, eliminates or restricts to very shallow water the background contrasting behavior. The data presented herewith and the pertinent references support such a correlation of light intensity and albedo with the locomotor activity and pigmentary behavior of fishes in reference to background. In a broader sense it would seem that these features of the behavior of the pigmentary system, together with the interacting nervous and endocrine systems, must have a very influential bearing on the evolution of pattern in fishes.

Summary

1. An entirely black *Trinectes lineatus* (Linnaeus) positioned itself conspicuously on a light sand background and showed a pattern of light cross bars only when the light intensity fell to a value too low for the human eye to see.

2. A *Trinectes inscriptus* (Gosse), with a network of fine lines, which was not notably conspicuously nocturnal, remained buried completely out of sight in the daytime.

3. An as yet untransformed *Trinectes lineatus*, taken in a tow net, developed pigment as its eye migrated and by the time this was complete the fish was fully pigmented.

4. Completely transparent and glass-like planktonic post-larvae of *Platophrys ocellatus* (Agassiz), with the eye transformation completed, on taking up residence on the same light sand did not develop pigment for a long time but nevertheless were practically invisible because of their glass-like transparency, but later when they did develop typical flounder background-matching color and pattern were just about as hard to find.

5. These apparently unorthodox pigmentary behavior patterns are discussed in reference to more usual modes and are found to be simply special or limiting cases, fully referable to known reactions and influences in the general behavior of teleost pigmentary systems.

Acknowledgements

The studies on which this paper is based have, for the most part, been carried out at the Lerner Marine Laboratory on Bimini in the Bahamas. I am indebted to Mr. Marshall Bishop for collecting some of the material and to Miss Carol Mosher for technical aid.

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EXPLANATION OF THE PLATES

Plate I. *Trinectes*

Fig. 1. *T. lineatus* (Linnaeus) in a typical pose and coloration on light sand in daylight. × 1½.

Fig. 2. The same fish in darkness. Picture made by means of prearranged photo-flash.

Fig. 3. *T. inscriptus* (Gosse) as seen against a background of the native sand in a pose never permitted by the fish under normal circumstances. × ⅔.

Fig. 4. *T. lineatus* before metamorphosis, showing the early development of extensive pigmentation, five days after hatching at a length of 2.5 mm.

Plate II. *Platophrys ocellatus* (Agassiz)

Fig. 1. An individual in the extremely transparent stage seen against a dark background with reflected light, showing the light pigmentation of the eyes and some very pale chromatophores. November 23, one day after capture.

Fig. 2. The same fish against a dark background with extremely oblique reflected light. November 24.

Fig. 3. The same fish by transmitted oblique light, partially against a dark background, showing the glass-like transparency. November 26. × 3½.

Fig. 4. The same fish by transmitted light so arranged as to bring out the pale chromatophores shown in Figure 1. Here they show as mere small smudges. November 27. Photographs by Carol Mosher.

Plate III. *Platophrys ocellatus* (Agassiz)

Fig. 1. The fish of Plate II and another larger individual against a background of light sand. This is the background normal to these fishes and on which they virtually disappear. They are here nearly transparent and the sand under them is plainly visible. The eyes of the smaller one can be found at the right of the picture, the fish facing up. Those of the larger are at the left, the fish facing down. December 12. × 1¾.

Fig. 2. The same fish. They are in the central portion of the picture. The smaller, to the right, is facing left, and the larger, to the left, is facing the lower left-hand corner. Slightly more pigment is present. December 14.

Fig. 3. The same fish, the larger showing full pigmentation and the smaller partial pigmentation. The smaller fish is to the left and facing a little to the right. The larger is to the right and facing a little left of upward. December 19. Photographs by Carol Mosher.

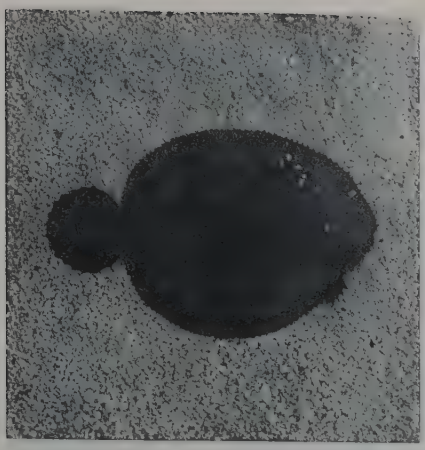

FIG. 1

FIG. 2

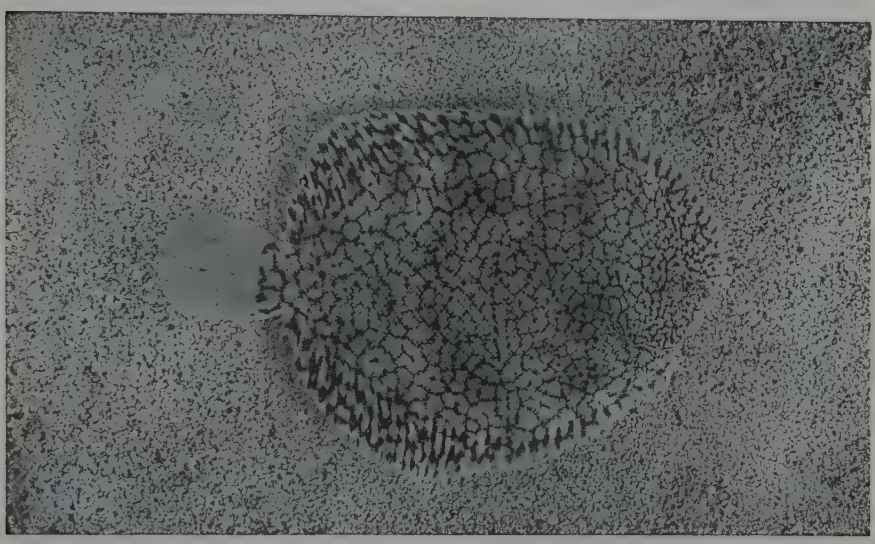

FIG. 3

FIG. 4

SPECIAL FEATURES OF VISIBILITY REDUCTION IN FLATFISHES

FIG. 1

FIG. 2

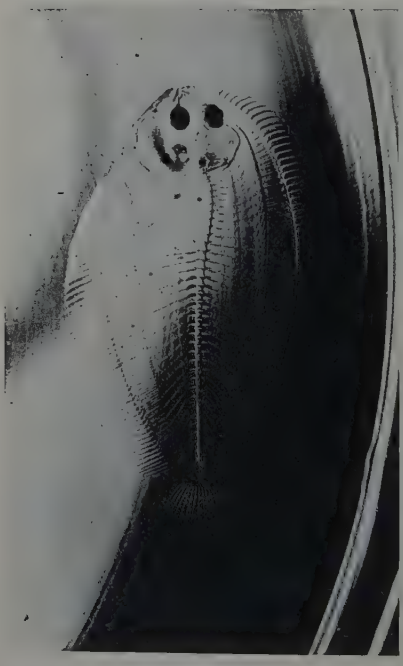

FIG. 3

FIG. 4

SPECIAL FEATURES OF VISIBILITY REDUCTION IN FLATFISHES

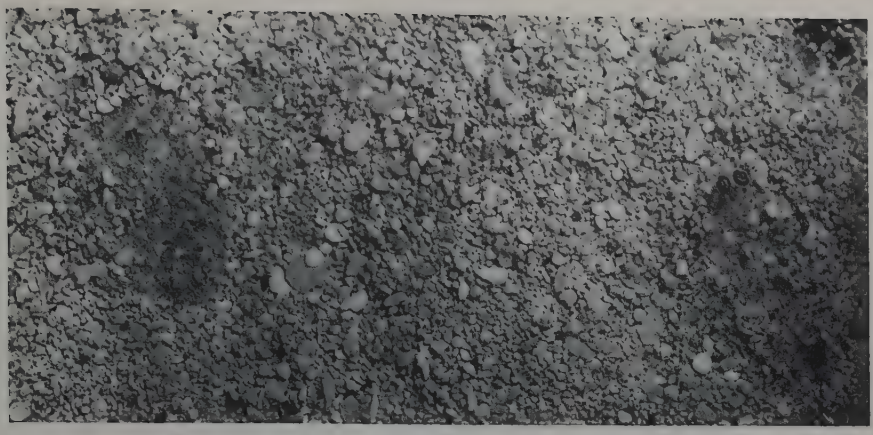

FIG. 1

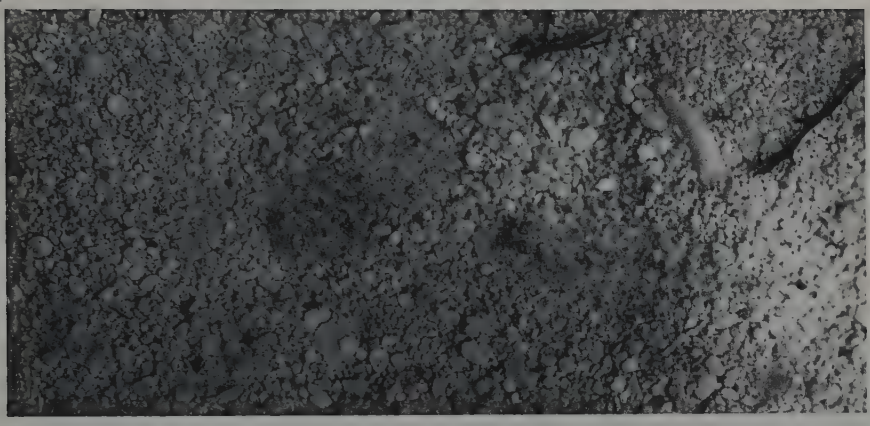

FIG. 2

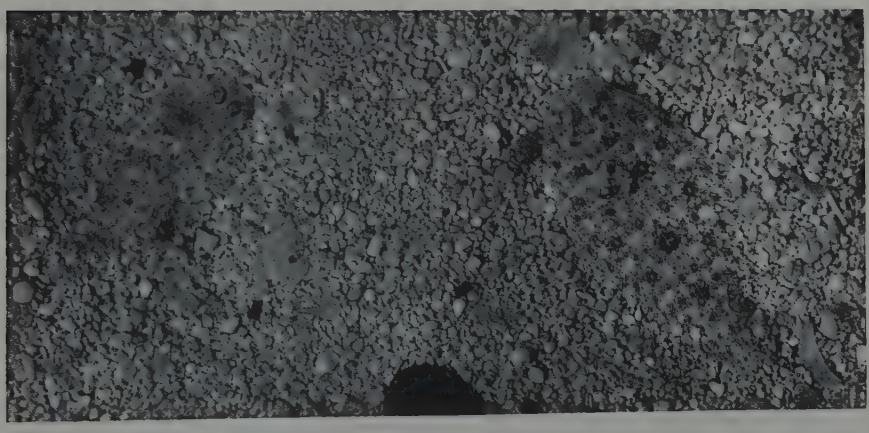

FIG. 3

SPECIAL FEATURES OF VISIBILITY REDUCTION IN FLATFISHES

10

A Comparative Study of the Morphology and Histochemistry of the Reptilian Adrenal Gland

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(Plates I-V; Text-figure 1)

INTRODUCTION

THERE is considerable evidence indicating the importance of the mammalian adrenal cortex in regulating certain aspects of protein, carbohydrate and electrolyte metabolism (Sayers, 1950). It would seem that any general concepts of adrenal histophysiology would be enhanced by comparative studies in all vertebrate classes. The function of the equivalent cortical tissue in the poikilothermous reptiles presents a challenging problem that has received little attention. Moreover, the morphology of the reptilian adrenal gland has not been adequately described for many common North American species. A basic knowledge of normal morphology should be a prerequisite for accurate interpretation of experimental modifications.

The purpose of this study is to describe the normal anatomy, histology, cytology and histochemistry of the adrenal glands of certain common North American representatives of the various orders of reptiles in order to determine the nature of the similarities and differences manifested by the adrenals. By this approach it was hoped that types especially suitable for future experimental studies would be discovered.

Knowledge of adrenal morphology in the Crocodilia is based primarily upon a brief anatomical and histological description of the adrenals of the alligator, *Alligator mississippiensis,* by Reese (1931). Lawton (1937), also, has described the gross anatomy and histology of the the alligator adrenal with special reference to the adrenal-autonomic complex and vascular supply. The histogenesis of the alligator adrenal has been described by Forbes (1940).

There is a paucity of information regarding the chelonian adrenal. Present knowledge is based principally on the early description of the adrenal in the turtle, *Emys orbicularis,* by Vincent (1898) and the description of the development of the adrenals in the loggerhead turtle, *Caretta caretta,* by Kuntz (1912).

The adrenals of the Serpentes have received little attention. Present knowledge is based mainly on the early descriptions of colubrid snakes by Vincent (1898), Minervini (1904) and Radu (1934); recent observations by Valle & Souza (1942) on the histology of the adrenals in the snake, *Dryophylax* sp.; and brief notes on the morphology of certain South American snakes by Uchoa Junqueira (1944).

The adrenal morphology of the Sauria has received some attention and histological descriptions are available for a few species of lizards. The first histological description of the reptilian adrenal is that of the European lizard, *Lacerta agilis,* by Braun (1882). Kraus (1921) gave a rather complete histological description of this species. Bimmer (1950) has described the seasonal variations in the adrenals of the closely related lizard, *Lacerta vivipara.* Vincent (1896, 1898) described the histology of the adrenals in *Chameleon vulgaris, Anguis fragillis, Lacerta agilis* and *Uromastix hardwickii.* More recently, Retzlaff (1949) described the histology of the adrenals in the alligator lizard, *Gerrhonotus multicarinatus.* Hartman & Brownell (1949) gave a brief description of the adrenals in the Gila monster, *Heloderma suspectum,* and the iguanid, *Anolis carolinensis.* Perhaps the most complete discussion of reptilian adrenal morphology is that by Miller (1952), who described the normal histology of the viviparous lizard, *Xantusia vigilis,* and its experimental modification by hypophysectomy, starvation and the ad-

[1]Accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy at New York University, June, 1954.

ministration of ACTH, Cortisone and DOCA; however, no histochemical studies were made.

The authors wish to express thanks to Drs. Ross F. Nigrelli and James A. Oliver of the New York Zoological Society, Mr. Charles M. Bogert of the American Museum of Natural History, and Dr. Royal B. Brunson of Montana State University, for specimens generously contributed for this study. Acknowledgement is also made to Mr. Albert H. Stenger for the photomicrography.

Materials and Methods

The reptiles used in this study were obtained from various sources. Young alligators, *Alligator mississippiensis,* from Schriever, Louisiana, were obtained through local animal dealers. Horned lizards, *Phrynosoma cornutum,* were obtained from the vicinity of Abilene, Texas. Skinks, *Eumeces fasciatus,* were obtained from the vicinity of Mena, Arkansas. Painted turtles, *Chrysemys picta,* were collected in the vicinity of Lakehurst, New Jersey, and were also obtained from collectors near Oshkosh, Wisconsin. Garter snakes, *Thamnophis sirtalis,* were collected in the vicinity of Glen Harbor, Long Island, New York, and supplemented by collections obtained from the vicinity of Oshkosh, Wisconsin.

A total of 304 animals was used in the study. These may be classified under the following orders, families and species, conforming with the recent checklist of North American reptiles (Schmidt, 1953):

Order Crocodilia
- Family Crocodilidae
  - 16 *Alligator mississippiensis* Daudin, Alligator
  - 10 *Caiman crocodilus* Linnaeus, South American Caiman

Order Chelonia
- Family Emydidae
  - 36 *Chrysemys picta* Schneider, Eastern Painted Turtle
  - 12 *Pseudemys scripta* Schoepff, Terrapin
  - 4 *Terrapene carolina* Linnaeus, Box Turtle
  - 4 *Clemmys guttata* Schneider, Spotted Turtle
- Family Chelydridae
  - 6 *Chelydra serpentina* Linnaeus, Snapping Turtle
- Family Kinosternidae
  - 4 *Kinosternon flavescens* Agassiz, Mud Turtle
  - 2 *Sternotherus odoratus* Latreille, Musk Turtle
- Family Trionychidae
  - 4 *Trionyx ferox* Schneider, Soft-shelled Turtle

Order Sauria
- Family Iguanidae
  - 36 *Phrynosoma cornutum* Harlan, Horned Lizard
  - 12 *Anolis carolinensis* Voigt, Green Anolis
  - 12 *Sceloporus undulatus* Latreille, Fence Lizard
- Family Scincidae
  - 24 *Eumeces fasciatus* Linnaeus, Five-lined Skink
  - 6 *Eumeces obsoletus* Baird & Girard, Great Plains Skink
  - 12 *Lygosoma laterale* Say, Little Brown Skink
- Family Gekkonidae
  - 1 *Tarantola mauretanica* Say, European Gecko
  - 1 *Gonatodes fuscus* Hallowell, Yellow-headed Gecko
  - 1 *Sphaerodactylus cinereus* Wagler, Ashy Gecko
  - 4 *Hemidactylus turcicus* Linnaeus, Turkish Gecko
- Family Anguidae
  - 12 *Gerrhonotus coeruleus* Wiegmann, Alligator Lizard
  - 6 *Ophisaurus ventralis* Linnaeus, Eastern Glass Snake
- Family Teidae
  - 8 *Cnemidophorus tessellatus* Say, Checkered Race Runner

Order Serpentes
- Family Colubridae
  - 48 *Thamnophis sirtalis* Linnaeus, Eastern Garter Snake
  - 6 *Natrix sipedon* Linnaeus, Common Water Snake
  - 6 *Lampropeltis doliata* Lacépède, Eastern Milk Snake
  - 6 *Opheodrys vernalis* Harlan, Smooth Green Snake
- Family Crotalidae
  - 2 *Crotalus viridis* Rafinesque, Prairie Rattlesnake
- Family Boidae
  - 2 *Boa constrictor* Linnaeus, Boa Constrictor
  - 4 *Charina bottae* Blainville, Rubber Boa

The animals, upon arrival in the laboratory, were kept in large vivaria, given ample water and supplied with food. Every attempt was made to utilize them as soon as possible after arrival, and only those specimens appearing to be in a healthy condition were used for histological study. The animals were anesthetized by intraperitoneal injections of sodium Nembutal in a 1:100 solution in 0.9% saline. The adrenals and immediately-surrounding tissues were rapidly removed and placed in fixative. Routine fixation fluids included 10% neutral formalin, Bouin's and Zenker-formal.

Tissues for general histological study were dehydrated and cleared by the dioxane method (Lillie, 1948). The tissues were then imbedded in 56-58° C. Tissuemat, serial sectioned at 5, 7 and 10 microns and the sections attached to slides with Mayer's albumen glycerol (Lillie, 1948). The following stains were used routinely: Mayer's acid hemalum and eosin, Masson's trichrome stain and Heidenhain's modification of Mallory's azocarmine-aniline blue technique (Lillie, 1948).

The distribution of connective tissue fibers was studied by a number of methods. Collagen was demonstrated by the Masson trichrome stain and the Van Gieson picro-acid fuchsin collagen method (Lillie, 1948). Weigert's iron hematoxylin was used as a nuclear stain with both methods. Elastic fibers were stained by Verhoeff's elastic tissue stain (McClung, 1952). To demonstrate reticulum, the Foot modification of Hortega's silver carbonate method was employed (McClung, 1952). Nerve fibers within the adrenal gland were stained by the Pearson-O'Neill (1946) silver-gelatine method following Bouin fixation.

For the study of mitochondria, tissues were fixed either in Regaud's fluid for 4 days and post-chromated for 7 days in 3% potassium dichromate (Lillie, 1948), or by Zenker-formal fixation followed by post-chromation for 48 hours at 37° C. in 3% potassium dichromate. The tissues were then dehydrated in graded alcohols, cleared in cedarwood oil and transferred through xylol to paraffin. Sections were cut at 3 microns. Mitochondrial stains used included Regaud's hematoxylin, Heidenhain's iron hematoxylin and aniline-acid fuchsin-methyl blue (Lillie, 1948).

Histochemical studies of cortical lipids were carried out on tissues fixed for 3 days in 10% formal-calcium (Baker, 1944). The tissues were then embedded in gelatin (Zwemer, 1933) and sectioned on the freezing microtome at 10 and 15 microns. The sections were attached to gelatinized slides as recommended by Baker (1946) and stored in Baker's formal-calcium-cadmium preservative. Histochemical tests were applied to the sections within a two-week period after the time of fixation. Prolonged storage in formalin resulted in a diffuse background staining.

The sudanophilic lipids of the cortical cells were demonstrated by Sudan black B in propylene glycol without counterstaining (Pearse, 1952). Cholesterol and its esters were demonstrated by the Shultz test (Lillie, 1948). The distribution of carbonyl-containing lipids was investigated by means of the Schiff leucofuchsin reagent (Lillie, 1948).

Observations

A. Crocodilia

(a) *Alligator mississippiensis*

Anatomy

The adrenals of the young alligator, *Alligator mississippiensis*, are elongate bodies located in a retroperitoneal position on the midventral surface of the kidneys and extending slightly anterior to the latter with the right adrenal extending slightly more cephalad than the left. They are in close association with the ventrally situated gonads and the laterally placed gonaducts. The adrenal gland is a discrete structure, separated from the kidney and reproductive organs by a distinct connective tissue capsule. The medial surface of the adrenals borders on the post caval vein (Fig. 1).

In shape, the adrenals appear ellipsoid in longitudinal view and ovoid-to-round in cross-section. The surface of the gland is smooth. In animals ranging in body length from 60 to 75 cm., the glands vary in size from 10 to 12 mm. in length and from 2 to 3 mm. in width. They can be distinguished from the gonads and kidney by their pinkish-white to yellowish-orange color.

The vascular supply consists of both arterial and venous blood. Several adrenal arteries branch off each side of the dorsal aorta and ramify over the dorsal surface of the gland in the connective tissue capsule. Small arterioles branch off and penetrate the parenchyma of the gland, finally connecting with venous sinuses. Arising from the dorsal body wall lateral to the midline, a medium-sized afferent vein enters the anterior dorsal surface of the adrenal and finally branches into a capsular network. The right adrenal is in intimate contact with the vena cava with a large central venous sinus of the gland opening directly into the vena cava through a series of openings or ostia in the wall of the vena cava. The left adrenal gathers blood into a large ventro-mesial capsular vein which empties into the vena cava near the center of the adrenal gland (Fig. 1).

The adrenal is innervated from the paired lateral sympathetic ganglia in the region of the

kidney. Sympathetic nerve fibers from at least four sympathetic ganglia supply each adrenal, thus producing a more or less segmented innervation. Large nerves consisting principally of unmyelinated fibers ramify through the pericapsular region (Fig. 2). In frozen sections stained with Sudan black B, thin rings of sudanophilic material are often noticed in the nerve fiber bundles. The nerves appear to be encapsulated in a sheath of collagenous fibers. Sympathetic ganglion cell clusters are located on the dorsal side of the gland in the connective tissue capsule. Following silver impregnation, a network of argyrophilic fibers can be distinguished. Coarse nerve fibers tend to follow small blood vessels toward the center of the gland and then branch out to form networks over the surface of cortical cords and medullary cell masses. From these networks fine thread-like fibers branch off and penetrate between the cortical and medullary cells, terminating as very fine threads.

Histology

The stroma of the adrenal consists of a distinct connective tissue capsule composed of collagenous and reticular fibers. The collagenous fibers are continuous with the surrounding connective tissue while the reticular fibers are more or less limited to the inner zone of the capsule and are continuous with the parenchymal reticulum. An inner parenchymal meshwork of reticular fibers supports the cortical cords and medullary cells (Fig. 3). While collagenous fibers are the predominant type found in the capsule, occasional short elastic fibers are noticed in the walls of capsular veins. No muscle tissue, other than that occurring in the media of small arteries, is present. Infrequent bundles of collagenous fibers form trabeculae which penetrate into the center of the gland carrying arterioles and nerve fibers. Fine collagenous fibers course between the parenchymal cell cords as well as in the walls of the vascular sinuses. Heavy reticular fibers in the inner layers of the capsule extend inward and provide an extensive network to support the cortical cells and medullary masses. Coarse fibers, stretching lengthwise between the cortical cords, give off a network of fine reticular fibers at right angles which enclose the cortical cords and medullary cell masses in a reticular framework (Fig. 3).

The alligator adrenal is a composite endocrine gland composed of cortical and medullary cells. The medullary tissue consists of a peripheral layer that encircles the cortical tissue together with small clumps and strands of medullary cells scattered throughout the interspaces between the cords of cortical cells and the vascular sinuses (Fig. 2). Intertwining and anastomosing solid cords measuring from 25 to 35 microns in diameter constitute the cortical tissue. Occasional cortical cords extend through the peripheral medullary tissue and touch upon the capsular connective tissue. Small nodules of undifferentiated cortical cells are often found scattered in the capsule. An intricate network of capillaries and venous sinuses pervades the cortical and medullary tissue.

The zonation typical of the mammalian adrenal is lacking in the alligator adrenal. The peripheral cords tend to be made up of elongated cells in a more or less radial arrangement while the central region of the gland consists of cords made up of irregularly dispersed polyhedral cells having centrally placed nuclei. These central cords often appear to be oriented toward the side of the gland bordering on the vena cava (Fig. 1). The venous sinuses are lined with an endothelium having flattened granular nuclei.

Cytology

The medullary tissue is composed of two cell types with different tinctorial and morphological characteristics. These differences are best observed after Zenker-formal fixation and staining with Masson's or with Heidenhain's azan stain. A peripheral zone of yellow-staining cells can be readily distinguished from the more centrally situated reddish-brown cells (Fig. 2). The yellow-stained cells will be referred to as the "light" cells and the reddish-brown cells as "dark" cells. The "light" cells are 15 to 18 microns in diameter with a centrally located nucleus measuring 5 to 7 microns and are spherical to polyhedral in shape. These "light" cells have a finely granular cytoplasm and occur singly, in small clumps and strands, and in rather large masses in the peripheral regions of the adrenal (Fig. 2). The majority of them are restricted to the capsular region; however, occasional strands extend inward among the cortical cords, especially in the connective tissue trabeculae penetrating the gland. The "light" medullary cells are often found in association with sympathetic ganglion cells in the capsule or in the adjacent connective tissue.

The "dark" cells occur singly or in small clumps or strands in the spaces and sinuses which pervade the cortical tissue (Figs. 2 & 5). This close association of "dark" medullary cells with the cortical tissue is strikingly evident in all tissues examined. These cells are, as a rule, smaller in size, measuring from 10 to 12 microns, and are generally polyhedral in shape. The granules in the cytoplasm are coarser and appear reddish-brown after staining with Masson's. The nucleus measures from 4 to 5 microns in diameter and is spherical, with 1 or 2 nucleoli.

In sections, the cords of cortical cells appear as spherical-to-elongate anastomosing masses of cells usually consisting of 1 to 4 tiers of cells (Fig. 2). The shape of the cells in the cord varies from irregular to greatly elongated spindle-shaped or wedge-shaped. The latter configurations are probably due to compression forces, since they are found more often in the tips of peripheral cords abutting against the capsule. The nuclei are centrally located, measuring 5 to 6 microns in diameter, and usually possess only one large fuchsinophilic nucleolus. Following Zenker-formal fixation and Masson's or Heidenhain's azan stain, the cytoplasm has a slightly fuchsinophilic and finely reticulated appearance.

Mitochondria are best demonstrated after post-chromation of Zenker-formal fixed material and staining with aniline-acid fuchsin and methyl blue. The small, granular, red-staining mitochondria are scattered throughout the cytoplasm (Fig. 5). The amount and distribution of mitochondria varies with the lipid concentration in the cortical cells. Lipid-poor cells exhibit a strong fuchsinophilia with the mitochondria concentrated in the perinuclear zone. Lipid-rich cells have granular mitochondria dispersed in the cytoplasmic matrix between the lipid vacuoles. One to several relatively large fuchsinophilic spheres are often seen in the cytoplasm in the vicinity of the nucleus.

In Baker's formal-fixed frozen sections stained with Sudan black B, the medullary cells and surrounding tissue remain colorless in contrast to the intense blue-black staining of the cortical cells (Fig. 4). The sudanophilic lipids are evenly distributed throughout the cortical tissue. Under high magnification the sudanophilic droplets appear to be more or less evenly distributed throughout the cytoplasm. There are differences in the amount of sudanophilic substances present: small undifferentiated cells possess little sudanophilic material, whereas other cells (particularly in peripheral cords) are filled with sudanophilic droplets.

Frozen sections subjected to the Shultz cholesterol test develop an intense blue-green color in the cortical tissue only. Medullary tissue, connective tissue and kidney tissue give a negative reaction to this procedure. The Shultz-positive droplets have a uniform distribution throughout the cytoplasm of the cortical cells, similar to the sudanophilic droplets. An intense and selective staining of frozen sections is observed upon their treatment with Schiff leucofuchsin. A purplish-red color develops throughout the cortical tissue. Except for the interesting observation that the elastic fibers of blood vessels stain a purple color, all other tissues in the sections are Schiff-negative. The distribution parallels that of the sudanophilic lipids.

(b) *Caiman crocodilus*

The animals used in this study were very young specimens, still having an internal remnant of the yolk-sac and a visible umbilical scar on the abdomen. The adrenal of the immature caiman is similar in anatomical and histological features to the adrenal of the alligator. The only significant difference noted was in connection with the cortical tissue. The cortical cells are not as well differentiated in the center of the gland as are the peripheral cells. The centrally located cells are small and polyhedral and irregularly arranged in cords, while those at the periphery are of a tall columnar type.

B. Chelonia

1. Family Emydidae

(a) *Chrysemys picta*

Anatomy

The adrenal glands of the painted turtle, *Chrysemys picta*, are paired bodies, yellowish in color, extending along the ventro-mesial surface of the kidneys in a retroperitoneal position (Fig. 6). They have an irregular outline, often appearing to consist of partially-fused spherical or irregularly-shaped masses imbedded around the numerous efferent renal veins. The gland is not completely encapsulated, but there is a close association and often an intermingling of renal tissue and adrenal tissue on the dorsal side of the adrenal. The ventral surface of the gland is covered by the pleuroperitoneum which, at the ventro-lateral edge, gives rise to the mesentery supporting the gonad.

There is an interesting difference in the anatomical relationships of the kidney, adrenal and gonad in the turtle, when compared with those of the alligator. The gonad of the young alligator is attached to the ventral surface of the adrenal, whereas the gonad in the turtle is separate and suspended in a mesenteric fold of the pleuroperitoneum (Figs. 1 & 6).

The arterial blood supply consists of several small adrenal arteries branching off from the dorsal aorta, as well as small branches arising from the genital and renal arteries that border or penetrate through the adrenal. Because of the unique location of the turtle adrenal with respect to the blood vessels of the kidney, it is difficult in histological sections to determine the identity of the veins and venous sinuses penetrating through the parenchyma of the gland. Doubtless, most of the sinuses represent efferent renal sinuses; however, some may be either sinuses of the renal portal veins or sinuses of afferent adrenal veins, all of which constitute a complex venous plexus in the region of the adrenal.

Numerous sympathetic nerve ganglia are found in the turtle adrenal, occuring most often in the connective tissue between the adrenal and the kidney or in the trabeculae which penetrate into the adrenal (Fig. 8). Following the silver-gelatine technique, a dense network of nerve fibers can be distinguished surrounding the medullary and cortical masses. A heavy network of fibers encircles and penetrates among the medullary cells, while in contrast only occasional fine, faintly-stained fibers penetrate the cortical tissue.

Histology

The stroma of the turtle adrenal is similar to that described for the alligator, except that in the turtle the gland is not completely encapsulated. A distinct capsule composed of collagenous and reticular fibers covers the ventral surface. Numerous trabeculae of collagenous and reticular fibers extend into the parenchyma, often appearing to separate the gland into separate masses of tissue. The columns and clumps of cortical and medullary tissue are outlined by a dense network of reticular fibers which serve as a framework to maintain the structural unity of the gland. Elastic fibers are limited to the adventitia of arteries and veins occurring in the capsule and trabeculae.

The adrenal of the painted turtle consists of intermixed cortical and medullary tissue (Fig. 7). The medullary tissue is not concentrated in any particular region of the gland, but is irregularly distributed among the cortical tissue as clusters of medullary cells. These clusters are of irregular size and shape, varying from small groups containing a few cells to large clumps and strands. It often appears that these clusters and strands lie in the vascular sinuses (Fig. 7).

The cortical tissue is composed of anastomosing and intertwining columns varying in width from approximately 40 to 70 microns. The columns are made up of several tiers or rows of cells which usually have an irregular arrangement (Figs. 7 & 8). Sometimes the cells in peripheral cords in longitudinal section have the appearance of being compressed into a single row of greatly elongated and flattened cells (Figs. 7 & 10).

Cytology

The medullary tissue consists of two cell-types which can be distinguished principally by their staining properties (Fig. 7). The peripheral medullary cells, after Zenker-formal fixation and Masson's stain, have a light brownish-yellow color in contrast to the darker yellowish-brown cells found in the more central region of the gland. The "light" cells occur singly or in small clumps or strands in the peripheral capsular region, and sometimes extend for a short distance in toward the center of the gland in the intercortical spaces, especially in trabeculae that penetrate the gland. The "dark" cells occur singly or in small clumps or strands either imbedded in the side of cortical cords or in the intercortical spaces and sinuses.

A striking histological feature of the "dark" cells is the fact that they are usually in close contact with cortical tissue. On the other hand, the "light" cells are more often separate from the cortical tissue, either in the peripheral capsule or in the surrounding connective tissue. They are also found in association with sympathetic ganglia (Fig. 8), in the adventitia of the dorsal aorta and in the parenchymal connective tissue of the kidney. Both cell types are polyhedral-to-spherical in shape, varying from 9 to 12 microns in diameter. The nucleus varies in size from 5 to 7 microns.

The cortical cells are variable in size and shape. The most common morphological type is an irregularly-shaped polyhedral cell (Figs. 7 & 10). Sometimes, due to compression forces on the column, the cells assume a tall, columnar-to-spindle-shaped form (Fig. 10). The centrally located nuclei are 5 to 6 microns in diameter, are slightly reticulated and usually have a single large hyaline nucleolus. Fuchsinophilic spheres are commonly found in a juxtanuclear position.

The mitochondria appear as small granules scattered throughout the cytoplasm, with the number of mitochondria depending upon the amount of lipid in the cell (Fig. 10). Highly vacuolated cells have few mitochondria, whereas cells having little lipid are mitochondria-rich. Only an occasional mitotic figure was noted in the cortical cells. The lack of any concentration of undifferentiated cells in the columns and a random dispersion of mitotic figures suggests that cell replacement must take place within the cords in a random fashion.

The cortical cell lipids are sudanophilic, Shultz-positive and Schiff-positive, with a uniform and parallel distribution throughout the cortical tissue. Under high magnification, the sudanophilic droplets appear to be evenly distributed throughout the cytoplasm and no zonation of the gland is seen (Fig. 9).

(b) *Pseudemys scripta, Clemmys guttata, Terrapene carolina*

The adrenals of the other members of the family Emydidae included in this study, namely, *Pseudemys scripta, Clemmys guttata* and *Terrapene carolina,* resemble (both anatomically and histologically) the descriptions for *Chrysemys picta.* The only significant histological difference is found in the adrenals of the spotted

turtle, *Clemmys guttata.* In this species there is considerably more medullary tissue. In transverse sections, the intermingling clumps and strands of medullary and cortical cells appear to be approximately equal in size and number.

2. Family Chelydridae

The adrenals of the snapping turtle, *Chelydra serpentina,* show no significant morphological differences from the description of *Chrysemys picta.*

3. Family Kinosternidae

The morphology of the adrenals of the mud turtle, *Kinosternon flavescens,* and the musk turtle, *Sternotherus odoratus,* resembles that of *Chrysemys picta* in general features. The glands in transverse sections appear more elongated and closely associated with the kidneys. The medullary tissue is often located in the adjacent renal tissue as yellow clumps among the renal tubules. The cortical cells show a tendency toward a regular arrangement in cords measuring approximately 50 microns in diameter. The cells are tall, columnar in shape and usually radially arranged in the cords so that in longitudinal sections they appear as a double row of cells. The nuclei are usually located near the end of the cell distal to the vascular sinus.

4. Family Trionychidae

The adrenals of the immature soft-shelled turtle, *Trionyx ferox,* resemble the adrenals of immature *Chrysemys picta* both in anatomical and histological features. In young animals such as these, the cortical cells are grouped in narrow cords and are not well differentiated because they have little cytoplasm or lipid material.

C. Sauria

1. Family Iguanidae

(a) *Phrynosoma cornutum*

Anatomy

The adrenal glands of the horned lizard, *Phrynosoma cornutum,* are paired bodies located near the anterior ends of the gonads and enclosed in the mesorchia or mesovaria supporting the gonads. In the male, the adrenals are intimately associated with the sperm ducts (Fig. 11). The right adrenal is slightly cephalad to the left, extending along the vena cava to the right lobe of the liver.

The glands are elongate, oval-shaped bodies measuring approximately 2 to 3 millimeters in length and 1 millimeter in width. The combined weight of the freshly-dissected glands in adult animals is approximately 1 milligram.

The blood supply consists of both arterial and venous blood. Each adrenal receives a branch from the dorsal aorta. These branches arise from the aorta at the origin of intervertebral arteries. In some specimens, the left adrenal receives two arteries from the aorta. A small afferent vein arises from the dorsal body wall cephalad to the adrenal and enters the anterior pole of the gland. The left adrenal is in contact with the cephalad end of the left efferent renal vein into which blood from the gland drains directly. The right adrenal is situated at the junction of the efferent renal veins and anteriorly along the vena cava. Blood from this gland drains directly into the vena cava.

Histology

The adrenal gland is enclosed in a thin connective tissue capsule composed of an outer layer of collagenous fibers which are continuous with the surrounding connective tissue, and an inner layer of reticular fibers which are continuous with the reticular framework supporting the parenchyma of the gland. Numerous trabeculae containing collagenous fibers and reticular fibers penetrate the parenchyma (Fig. 12).

Sympathetic ganglion cells are aggregated principally in the dorsal region of the capsule near the adrenal artery (Fig. 12). Occasional sympathetic cells are found in association with groups of medullary cells in other regions of the capsule. The adrenal gland of the horned lizard consists of cortical and medullary tissue arranged in a typical saurian pattern (Fig. 12). The medullary component comprises an incomplete dorsal capsule of primarily "light" type medullary cells with a few strands often extending inward from the periphery. In addition, small groups and individual cells of the "dark" type are interspersed in the parenchyma.

The cortical tissue consists of interwining and anastomosing cords measuring 30 to 50 microns in diameter (Fig. 12). In transverse section, a typical cord appears as a radially arranged group of 15 to 20 tall columnar cells with very indistinct cell outlines. The nuclei are regularly arranged in a zone near the periphery of the cord. In longitudinal section, a cord often presents the characteristic appearance of a double row of tall columnar cells with nuclei arranged in a double row near the peripheral edge of the cord. The central area of the cord, following Masson's staining, has a pale fuchsinophilic vacuolated appearance.

The cephalic pole of the right adrenal is often fused with the caudal tip of the dorsal lobe of the liver, both tissues being in contact with and partially encapsulating the vena cava in this region. The liver parenchyma is not distinctly separated from the cortical tissue by a connective tissue capsule and as a result small groups

of cortical cells in many sections appear to be isolated in the liver parenchyma.

Cytology

The medullary tissue consists of two cell-types (Fig. 12). The strands and masses of medullary cells in the peripheral capsular region are of the "light" type, having few granules and appearing light-yellow after Zenker-formal fixation. The medullary cells in the central part of the parenchyma are of the "dark" type, staining a reddish-brown after the Masson or azan technique. Both cell types are similar in their morphology, consisting of polyhedral cells which are 9 to 12 microns in diameter. The centrally located nucleus varies from spherical to oval in shape and measures 4 to 5 microns in diameter and has a granular appearance with one or more nucleoli. Granular mitochondria are dispersed throughout the cytoplasm.

The anastomosing cords of cortical tissue are composed of polyhedral cells of varying height (Fig. 12). The most frequently observed type is a tall, wedge-shaped columnar cell approximately 20 microns in height and 7 microns in width at the base. The spherical-to-oval-shaped nucleus is 4 to 5 microns in diameter and contains one or more nucleoli. Generally, only a single large fuchsinophilic nucleolus is present.

The mitochondria in the cortical cells appear as fine granules, either concentrated in the perinuclear zone or dispersed in the delicate cytoplasmic network surrounding the lipid vacuoles. The lipid droplets are evenly distributed throughout the cytoplasm and give a strong, positive color reaction to the Sudan procedure (Fig. 13). There is a general and parallel distribution of sudanophilic lipids, carbonyl lipids and cholesterol esters.

(b) *Anolis carolinensis*

The adrenals of the green anolis, *Anolis carolinensis,* are similar in anatomical features to *Phrynosoma* (Fig. 14). The histological picture differs somewhat in that the cortical cords appear to be narrower (25 to 35 microns) and are made up of radially arranged low columnar cells. The nucleus is centrally located or may, in some cords, be located near the edge of the cell bordering on the blood supply. Fewer medullary cells are seen in the parenchyma of the gland with most of them being concentrated on the dorsal surface of the gland.

(c) *Sceloporus undulatus*

The adrenals of the fence lizard, *Sceloporus undulatus,* resemble, in general, those of *Anolis.* In this species the cortical cells are of a low columnar type with a tendency toward exhibiting a polarity by the display of nuclei at the peripheral edges of the cortical cells.

2. Family Scincidae

Anatomy

The anatomical relationships of the adrenals in the three species of skinks that were studied, namely, *Eumeces fasciatus, Eumeces obsoletus* and *Lygosoma laterale,* are in general similar to the saurian pattern described previously for the iguanid lizard, *Phyrnosoma.* The glands are elongate bodies closely associated with the gonads and gonaducts. The right adrenal in this group of lizards does not make contact or fuse with the dorsal lobe of the liver; however, the mesentery of the right gonad is continuous with the mesentery supporting the dorsal lobe of the liver. A single afferent adrenal vein enters the caudal pole of each adrenal. A large sympathetic ganglion is located near the cephalic pole of each adrenal.

Histology

The gland is enclosed in a thin capsule continuous with the surrounding mesentery and connective tissue. The parenchyma of the gland is supported by a network of thick reticular fibers.

The disposition of the cortical and medullary tissue corresponds to the saurian pattern described for *Phrynosoma.* The medullary cells form a partial capsule around the dorsal surface of the cortical tissue. Some medullary tissue in the form of individual cells and small clumps is diffused throughout the parenchyma. The medullary cells are of two tinctorial types: the "light" cells which are confined chiefly to the periphery; and the deeper-lying cells which are usually of the "dark" type.

The arrangement of the cortical cells within the cords is a unique and significant feature in the Scincidae (Figs. 18 & 23). In transverse section, a cord consists of 10 to 12 tall, wedge-shaped columnar cells arranged in a radial pattern with the nuclei abutting against the peripheral edge of the cell. In longitudinal section, the cord appears to consist of two rows of tall columnar cells with parallel rows of nuclei at the edges of the cord. This marked polarity of the cortical cells is present in all three species of skinks examined. Occasionally, a lumen is observed in the center of a cord, giving the cord an appearance that resembles an exocrine gland. These central cavities are not a consistent feature and probably are artifacts resulting from shrinkage.

Cytology

The cortical cells display a marked polarity with the nucleus abutting against the edge of the cell facing the vascular supply. The region of the cell distal to the nucleus contains a faintly-

staining cytoplasmic network surrounding lipid vacuoles of varying size. Following aniline-acid fuchsin and methyl blue, the red-staining mitochondria are found widely scattered in the cytoplasmic network (Fig. 19). The typical cortical cell is a lipid-rich, mitochondria-poor type. Undifferentiated cortical cells are not commonly seen in the capsule, which would seem to suggest that cortical cell replacement occurs within the individual cords and not by migration.

The unique polarity of the cortical cells is clearly demonstrated by histochemical tests of the lipid droplets. In frozen sections where a cord happens to be cut lengthwise, the blue-black sudanophilic lipids are confined to a central zone of the cord and the nuclear zone at the periphery fails to stain (Fig. 20). A similar pattern of distribution of Shultz-positive and Schiff-positive droplets is found. No significant difference in the uniformity of staining is apparent. The medullary and surrounding connective tissue do not give a positive reaction to any of the tests applied.

An intense blue-green color reaction is obtained by the Shultz test for cholesterol and its esters. The distribution of the blue-green droplets is localized in the cytoplasm distal to the nucleus. Following application of the Schiff leucofuchsin technique, an intense red color develops exclusively in the cortical tissue. The intensity and specific localization of the color reaction in the cytoplasmic region of the cortical cells indicates that the cortical tissue, following Baker's fixation, contains a high level of carbonyl lipids.

3. Family Gekkonidae

A limited number of specimens from this family was available for histological study. The series consisted of a single adult European gecko, *Tarantola mauretanica;* one adult yellow-headed gecko, *Gonatodes fuscus;* one adult ashy gecko, *Sphaerodactylus cinereus;* and four adult Turkish geckos, *Hemidactylus turcicus.* The anatomical relationships in these specimens resemble the general saurian pattern already described. The disposition of the cortical and medullary tissue is similar to that noted in other groups of lizards.

A special and significant feature of the gecko adrenal is the extreme polarity of the cortical cells (Figs. 21 & 22). The arrangement of the cells in the cords is similar to that described for the Scincidae. The cortical tissue, in transverse or longitudinal sections, appears as spherical-to-oblong anastomosing cords measuring approximately 50 microns in diameter. The cells in longitudinal sections of a cord appear as tall, wedge-shaped columnar cells with their nuclei abutting on the peripheral edges of the cells facing a capillary or venous sinus. Because of the extremely small size of these animals and the consequent small size of the gland, a typical transverse section appears to consist of 3 or 4 anastomosing cords.

A single specimen of the Dibamidae, *Dibamus novae-guineae,* and two representatives of the Pygopodidae, *Lialis burtoni* and *Pygopus lepidopus,* were examined histologically and found to have a gekkonid arrangement of the cortical cells.

4. Family Teidae

The anatomical features of the adrenals of the checkered race runner, *Cnemidophorus tessellatus,* conform to the saurian pattern already described for *Phrynosoma.* The microscopic anatomy, however, shows certain characteristics which are distinctive of this species (Fig. 17). The medullary tissue is more widely dispersed within the cortical tissue. In transverse sections, a dorsal aggregation is present, as well as numerous clumps scattered throughout the parenchyma of the gland. Both "light" and "dark" cells are present with the "light" cell type more or less restricted to the dorsal aggregation or to extra-adrenal locations.

The cortical cells do not exhibit a consistent regular arrangement, but generally appear to have a more or less random dispersion (Fig. 17). Occasional cords, 20 to 30 microns in diameter, present a radial and polarized arrangement of tall columnar cells with nuclei aligned on the periphery. Most of the cells are low columnar to polyhedral in shape, measuring approximately 10 microns in height and 7 microns in width. The nuclei are usually centrally located or are found toward the edge of the cell facing a vascular sinus.

5. Family Anguidae

(a) *Gerrhonotus coeruleus*

The morphology of the adrenals of the alligator lizard, *Gerrhonotus multicarinatus,* has been described by Retzlaff (1949). They exhibit the general saurian anatomical relationships observed for other lizards. The significant and specific characteristic feature of the anguimorphid type of adrenal is illustrated by *Gerrhonotus coeruleus.* The cortical cells are irregularly shaped and do not arrange themselves in definite cords or columns (Fig. 15). Only cortical cells bordering on a vascular sinus tend to arrange themselves in a regular fashion around the sinus with the nuclei near the side of the cell which faces the sinus. Most of the cells have centrally located nuclei. An unusual feature of the cortical tissue of *Gerrhonotus* is the presence in large individuals of a narrow peripheral zone of cor-

tical cells that stain less intensely than the rest of the cortical cells (Fig. 15). No histochemical tests were made on the few animals available to determine the nature of the lipids in this peripheral zone.

(b) *Ophisaurus ventralis*

The adrenals of the glass snake, *Ophisaurus ventralis,* exhibit the general saurian pattern. In this species, the body is greatly elongated like that of a snake, yet the adrenals and associated reproductive organs display only a slight asymmetry—the right gonad being slightly more cephalad than the left, but no more so than the usual disposition in the Sauria. The microscopic anatomy of this species is similar to the anguid lizard, *Gerrhonotus,* just described.

D. Serpentes

1. Family Colubridae

(a) *Thamnophis sirtalis*

Anatomy

The adrenals of the Eastern garter snake, *Thamnophis sirtalis,* are discrete bodies closely associated with the asymmetrically-located gonads (Fig. 24). They can be distinguished from the latter by their golden-yellow color and difference in texture. The left adrenal lies anterior to the left kidney near the cephalic end of the left efferent renal vein and extends anteriorly for a short distance alongside the gonad. With reference to the ventral scutes or gastrosteges, the left adrenal lies approximately between the 31st to 34th gastrostege in females and between the 35th to 37th gastrostege in males, counting anteriorly from the anal plate. The right adrenal lies alongside the vena cava just anterior to the junction of the efferent renal veins and is closely associated with the caudal end of the right gonad. The right adrenal lies approximately between the 45th and 48th gastrostege in both sexes. The glands are contained in the mesovaria or mesorchia supporting the gonads (Fig. 24). In the male, there is a close association with the vas deferens.

The adrenals are elongate bodies varying in length from 5 to 15 mm., depending on the body length of the snake. Usually the right adrenal is slightly longer than the left. Occasionally a gland appears to be constricted into a bilobed shape. In transverse section, the gland is elliptical-to-round with a diameter of 1 to 3 millimeters. Adrenal weights for a series of 13 garter snakes of mixed sex range from 0.03 to 0.08 per cent. of the body weight, with an average of 0.045 per cent.

The garter snake adrenal is an extremely vascular organ having both an arterial and a venous blood supply. The dorsal aorta gives off to each adrenal a branch (the spermatic or ovarian artery) which enters the mid-dorsal region of the gland, bifurcates, sending a branch anteriorly through the dorsal pericapsular connective tissue, and continues into the testes or ovary (Fig. 24). The posterior branch continues caudally through the dorsal pericapsular layer of the adrenal and finally joins the gonaduct arteries. Numerous arterioles branch off from these longitudinal arteries to enter the parenchyma of the gland.

A separate venous supply can readily be seen on gross examination. Two (sometimes only one) afferent veins emerge from the dorsal body wall slightly lateral to the midline and enter the mid-dorsal region of the gland. Usually the second vessel emerges from the body wall just posterior to the adrenal and joins a longitudinal vein in the mesotuberium which connects with the anterior branch from the body wall. Branches from this venous supply connect with the venous sinuses and capillaries in the parenchyma of the gland.

The blood from the left adrenal gland is carried by a number of small, extremely short, efferent adrenal veins opening into the left efferent renal vein. The blood from the right adrenal drains into the postcaval vein either by means of numerous short efferent veins or is so intimately associated with the vein that blood from the parenchymal sinuses drains directly into the vena cava through small openings (Fig. 24).

A large aggregation of sympathetic ganglion cells is located in the dorsal capsular region, usually near the artery. Occasional sympathetic nerve cells are seen in the capsule of the gland; however, they were not found in the parenchyma of the gland. The medullary cells are innervated by an extensive network of nerve fibers, although nerve endings to the cortical cells cannot be definitely distinguished in the silver carbonate preparations.

Histology

The garter snake adrenal, fully encapsulated by the connective tissue, is almost completely enveloped in the surrounding mesentery (Figs. 24 & 25). The capsule consists chiefly of collagenous fibers with a few fine reticular fibers in the innermost layers. The parenchyma of the gland is supported by a thick network composed primarily of collagenous fibers. Fine reticular fibers are found in the walls of the vascular sinuses, where they form a fine network enveloping the cords of cortical tissue.

The medullary and cortical tissues are disposed in a pattern somewhat similar to that of the lizards (Fig. 25). The greater part of the medullary tissue is concentrated in the dorsal

pericapsular region in the vicinity of the spermatic or ovarian artery. In transverse section, a large mass of "light" yellow cells is seen aggregated around the dorsally located artery. Medullary cells of the "dark" type are found scattered in and among the cords of cortical cells singly or in small groups of three or four cells (Figs. 25 & 28).

The cortical tissue exhibits a unique and characteristic arrangement (Figs. 25 & 28). The cortical cells are arranged in anastomosing and intertwining cylindrical cords measuring 40 to 50 microns in diameter. In transverse section, a cord appears spherical and to consist of 12 to 15 radially-disposed tall, wedge-shaped columnar cells. The nuclei are regularly arranged in a small circle in the center of the cord. In longitudinal section, a cord appears to consist of two rows of tall columnar cells with a double row of nuclei running lengthwise in the center of the cord. A common occurrence in the garter snake adrenal is the presence of strands and large masses of undifferentiated cortical cells in the capsule (Fig. 25). These masses of cells, if followed in serial sections, are often found to be continuous with the parenchymal cortical tissue. The undifferentiated cells have a very limited amount of cytoplasm which, after Masson's staining, appears strongly fuchsinophilic.

The garter snake adrenal is an extremely vascular gland and this is made apparent in histological preparations by the large and extensive network of vascular sinuses that pervades the gland (Fig. 25). These sinuses are lined with a thin endothelium having extremely flattened nuclei.

Cytology

Two tinctorial types of medullary cells are found in the garter snake adrenal. The cells aggregated near the dorsally-located artery have a yellow, finely-granular appearance after Zenker-formal fixation and Masson's stain. The small clumps and individual cells associated with the cortical tissue possess coarse granules and stain reddish-brown. Both types are generally polyhedral in shape and measure 12 to 14 microns in diameter.

The cortical cells are usually of a tall columnar type measuring 20 to 25 microns in height. There is neither notable sexual variation nor discernable seasonal difference in cell height in the series of garter snakes which was collected in April, July and October. The cells exhibit a marked polarity with the nucleus usually located near the end of the cell distal to the blood supply (Figs. 25 & 28). The nuclei are 4 to 5 microns in diameter, spherical-to-oval in shape, and exhibit from one to three prominent nucleoli.

The mitochondria are granular bodies occurring throughout the cytoplasm (Fig. 28). In those cells with a high lipid concentration, the mitochondria are few in number and widely scattered in the cytoplasm surrounding the lipid vacuoles. In the lipid-poor undifferentiated cells, a heavy concentration of mitochondria pervades the cytoplasm. There is an inverse ratio between the amount of mitochondria and lipid in the cortical cells. Fuchsinophilic spheres larger than mitochondria occur frequently in the cytoplasm.

An outstanding feature of the garter snake adrenal is the distribution of lipid inclusions in the cortical cells. The lipid droplets are osmiophilic, sudanophilic, Schultz-positive, and exhibit an intense positive reaction to the Schiff leucofuchsin reagent. The lipid droplets appear to be evenly distributed throughout the cortical tissue and no tendency toward zonation is observed (Fig. 26).

Within the cells, the lipid droplets are concentrated at the peripheral end of the cell and at the end opposite the nucleus. This polarity is strikingly demonstrated in frozen sections stained with Sudan black B (Fig. 26), or by the Shultz technique for demonstrating cholesterol and its esters (Fig. 27). A similar distribution of Schiff-positive carbonyl lipids is seen, there being a parallel pattern of distribution of the sudanophilic, Schultz-positive and Schiff-positive lipids. By chance, one of the frozen sections tested for cholesterol was observed to have a mass of undifferentiated cells in the capsule (Fig. 27). Only a slightly positive reaction was seen in this nodule.

(b) *Natrix sipedon*

The adrenals of the common water snake, *Natrix sipedon,* are in general similar in morphology to those of *Thamnophis.* Medullary tissue is aggregated in the dorsal region of the capsule and small groups of cells are scattered throughout the cortical tissue. The cortical tissue exhibits a regular arrangement of polarized columnar cells into anastomosing cords. The polarization is similar to that of the garter snake.

(c) *Lampropeltis doliata*

The morphology of the adrenals of the Eastern milk snake, *Lampropeltis doliata,* is similar to that of *Thamnophis* and *Natrix.*

(d) *Opheodrys vernalis*

The adrenals of the smooth green snake, *Opheodrys vernalis,* are similar in anatomical features to those of the other colubrid snakes mentioned. Histologically, however, the parenchyma of the gland is more compact. The cortical tissue is arranged in radial cords of low

columnar cells. The nuclei are located distal to the capillaries.

2. Family Crotalidae

Only two specimens of the prairie rattlesnake, *Crotalus viridis,* were studied. Both of these animals had been preserved in 10% formalin for an unknown period of time. The adrenals were poorly fixed, yet on staining with Mayer's acid hemalum and eosin, sufficient detail was present to warrant comment. Anatomically, the glands are similar to those described for the garter snake. Microscopically, a typical ophidian disposition of cell types is seen. The medullary tissue is confined primarily to the dorsal capsular region while the cortical cells are regularly arranged in anastomosing cords. The nuclei show a similar disposition and polarity to that noted for the garter snake.

3. Family Boidae

(a) *Boa constrictor*

The adrenals of the boa constrictor are similar, for the most part, to those of the colubrid snakes. There is a tendency toward an elongation of the right gonad and a consequent elongation of the right adrenal. The microscopic anatomy is the typical ophidian type with a dorsal cap of medullary cells plus small clumps and single cells scattered in the cortical tissue. The cortical tissue consists of anastomosing cords made up of radially arranged low columnar cells exhibiting a slight polarity. The nuclei are arranged near the center of the cords or distal to the blood sinuses.

(b) *Charina bottae*

The adrenals of the rubber boa, *Charina bottae,* are elongated thread-like glands located in the mesenteries supporting the gonads. The left adrenal measures approximately 10 millimeters in length and 1 millimeter in width, whereas the right adrenal is approximately 25 millimeters in length and 1 millimeter in width.

The vascular supply of these glands consists of a single artery to the left adrenal and three arteries to the right adrenal. Blood leaves the glands by a series of short efferent adrenal veins, 6 for the left adrenal and 12 for the right adrenal. The glands are not attached directly to the vena cava, as noted in other snakes, but both glands empty into the vena cava, the left adrenal lying just anterior to the junction of the efferent renal veins.

In order to determine the arrangement of the cortical and medullary tissue, histological preparations were made of glands removed from museum specimens. Formalin-fixed tissues such as these will, on staining with Weigert's iron hematoxylin, give fairly good differentiation of cortical and medullary cells. The medullary cells exhibit a strong basophilia and stain dark blue. The adrenal has a regular ophidian distribution of glandular tissue with a dorsal aggregation of medullary cells and the cortical cells arranged in cords. In sections the cords appear to be made up of several layers or tiers of cells.

Discussion

Anatomy

The adrenal glands of reptiles exhibit a consistent association with the reproductive organs and the venous system. These anatomical relationships are found in all four orders of the reptiles studied.

In the Crocodilia, the adrenals are discrete, elongate bodies located in a retroperitoneal position on the midventral surface of the kidney in close association with the ventrally situated gonads and gonaducts. This relationship has been described for the alligator (Pettit, 1896; Spanner, 1929; Reese, 1931; Lawton, 1937), and the caiman (Pettit, 1896), and is confirmed in the present study.

A consistent anatomical relationship is found in all the species of North American turtles examined in this study. The adrenals are located on the ventral surface of the kidneys in close association with the gonaducts and gonads. The latter, however, are separate from the adrenal, being suspended ventrally from the adrenal-kidney complex by the mesorchia or mesovaria. The adrenal is not as well organized into a discrete gland as is that of the Crocodilia, but, rather, usually consists of partially fused spherical-to--irregular-shaped masses intimately associated with the vascular plexus on the ventral surface of the kidney. This close relationship with the kidney resembles the condition found in the amphibians (Radu, 1931; Villee, 1943).

There are few anatomical descriptions of the adrenals of the Chelonia in the literature. Vincent (1896), Minervini (1906) and Thompson (1932) give brief descriptions of the genus *Testudo.* Ogushi (1911) describes their relationship to the kidneys in the soft-shelled turtle, *Trionyx japonicus.* Kuntz (1912) describes the embryonic development and gross anatomy of the adrenals in young specimens of the loggerhead turtle, *Caretta caretta.* The gross anatomical relationships in *Emys europaea* have been reported by Spanner (1929). These early descriptions have been reviewed by van der Sprenkel (1934). In the marine tortoise, *Caretta caretta,* the adrenals are usually united into a single structure, according to Holmberg & Soler (1942).

The adrenal glands in the Sauria are found

enclosed in the mesorchia or mesovaria supporting the gonads. This consistent relationship has been reported in the agamid lizard, *Uromastix hardwickii* (Vincent, 1896), the *Chamaeleon* sp. (Minervini, 1904), various iguanids (Brooks, 1906; Reynolds, 1938), in the Lacertidae (Braun, 1882; Pettit, 1896; Vincent, 1896; Krause, 1922; Radu, 1934), the teid lizard, *Cnemidophorus gularis* (Brooks, 1906), the anguimorph lizards, *Gerrhonotus* (Retzlaff, 1949), *Heloderma* (Hartman & Brownell, 1949) and members of the Varanidae (Pettit, 1896).

The adrenals of the Serpentes bear the same relationship to the reproductive organs as was noted in the lizards; however, in this group the elongation of the body has resulted in an asymmetrical placement of the paired internal organs. The right adrenal is always situated cephalad to the left. This arrangement has been observed in many colubrid snakes: *Natrix natrix* (Pettit, 1896; Spanner, 1929; Radu, 1934), *Coluber* sp. (Vincent, 1896; Minervini, 1904; Uchoa Junqueira, 1944), *Dryophylox* sp. (Valle & Souza, 1942), the garter snake, *Thammophis sirtalis* (Atwood, 1918) and the water snake, *Natrix sipedon* (Bragdon, 1953).

The relative position of the adrenals in *Thamnophis sirtalis* can best be correlated with the ventral scales, in which case a consistent relationship is noted. The left adrenal lies between the 35th to 37th gastrostege in males and between the 31st to 34th in females. In both sexes, the right adrenal lies between the 45th and 48th scute, counting anteriorly from the anal plate. This separation of approximately ten scutes has been reported also for the common water snake, *Natrix sipedon* (Bragdon, 1953).

While extensive data on adrenal weights were not obtained, the average determined— 0.045 per cent. of the body weight— agrees with the range of 0.04 to 0.05 per cent. reported for other colubrid snakes by Naccarati (1922).

Bourne (1936, 1949) states that the adrenals of the boa constrictor exhibit a primitive anatomical condition in that they are located along the lobes of the attenuated kidneys. This association with the kidneys, however, was not found in the specimens of the boa constrictor and the rubber boa, *Charina bottae,* that were examined in this study. Both species have a typical serpentine arrangement with the adrenals located along the greatly elongated and asymmetrically-placed gonads. This disposition of the adrenals has previously been reported for the boa constrictor by Poll (1905) and the python (Pettit, 1896; Beddard, 1906a, 1906b; Spanner, 1929). In the very young anaconda, *Eunectes notaeus,* Beddard (1906a) noted that the adrenals are associated with the persistent mesonephros but that this condition is not found in adult animals. This would suggest that Bourne probably based his descriptions on very young animals that still possessed mesonephros kidney tissue.

The vascular supply of the adrenals of reptiles consists of both an arterial and venous supply. In the Crocodilia, the arterial supply consists of several pairs of arteries which arise from the dorsal aorta and enter the dorso-mesial region of the capsule. This general picture has been described by Pettit (1896), Reese (1931) and Lawton (1937). The arterial supply of the adrenal in the Chelonia consists of several small paired adrenal arteries from the aorta, as well as small branches from the renal and genital arteries. Very few observations on the arterial supply have been reported in the literature. Thompson (1932) described the arterial supply to the adrenal in the tortoise, *Testudo graeca.*

The arterial supply to the adrenals in the Sauria exhibits some variation among the various species studied. In the horned lizard, *Phrynosoma cornutum,* each adrenal receives one (sometimes two) arteries from the dorsal aorta. These arteries also supply the gonads and perhaps should be referred to as the spermatic or ovarian arteries. The adrenals receive blood from branches arising from these arteries, as the former are in close contact with the adrenals. In *Anolis carolinensis,* separate arteries supplying the adrenal are present. Pettit (1896) reported finding three arteries supplying the adrenal in *Varanus niloticus* and only a single artery to each gland in *Varanus salvator.* Bhatia (1929) observed that branches of the spermatic arteries supply the adrenals of the agamid lizard, *Uromastix hardwickii.* According to Retzlaff (1949), the arterial adrenal blood in the alligator lizard, *Gerrhonotus multicarinatus,* is derived from branches from the aorta, renal and mesenteric arteries.

In the Serpentes, the adrenal arterial supply comes from branches of the spermatic or ovarian artery as it courses in an anterior-posterior direction along the dorsal region of the connective tissue capsule. This general pattern has been reported for *Natrix natrix* (O'Donoghue, 1912), for the rat snake, *Ptyas mucosus* (Ray, 1934), and previously reported for the garter snake, *Thamnophis sirtalis,* by Atwood (1918).

A unique feature of the vascular supply of the reptilian adrenal is the presence of an adrenal portal system. This system of vessels has been carefully studied by a number of morphologists and their observations have been reviewed by van der Sprenkel (1934). In the alligator and caiman, a single adrenal portal vein arises from the dorsal body wall anterior to the adrenal,

enters the anterior pole of the adrenal, and branches into a plexus connecting with the vascular sinuses in the parenchyma of the gland. This arrangement has been described by Pettit (1896), Lawton (1937) and Spanner (1929) and is confirmed in the histological preparations of this study.

In the Chelonia, Spanner (1929) pictures three adrenal portal veins branching off from the renal portal veins. The presence of a portal system in the turtles which were studied could not be definitely determined by the histological approach employed.

In the Sauria, a variable number of portal veins branch off from the dorsal body wall and enter the adrenals. Beddard (1904b, 1904c, 1905) and Spanner (1929) examined a number of species of lizards and noted considerable species variation in the number and relationship of the adrenal portal veins. In the present study, a single adrenal portal vein was observed entering the anterior pole of the adrenal of *Phrynosoma cornutum.* Retzlaff (1949) does not mention a portal supply for *Gerrhonotus* nor does Miller (1952) for *Xantusia.*

In the Serpentes, the adrenal portal system is well developed. For example, in the garter snake, *Thamnophis sirtalis,* two relatively large veins enter each adrenal from the dorsal body wall. From one to three veins have been reported entering the snake adrenal (O'Donaghue, 1912; Ray, 1936; Beddard, 1904a, 1904d, 1906a).

According to Beddard (1906a), the adrenal portal veins in reptiles are persistent parietal branches of the postcardinal veins associated with the embryonic mesonephros kidneys. Their adrenal portal relationship is a secondary function taken over in the mature animal.

It is interesting to note that the adrenals of reptiles are always located in the region where the efferent renal veins join to form the inferior vena cava. In the Chelonia, both adrenals are in intimate contact with the efferent renal veins. In the Crocodilia, the adrenals are associated with the vena cava, the right adrenal being more intimately joined to the vena cava than the left. In the Sauria and Serpentes, the left adrenal usually drains either via short efferent veins or directly into the anterior portion of the left efferent vein while the right adrenal usually drains directly into the vena cava.

The nerve supply to the adrenal is derived from lateral sympathetic ganglia, the extent of innervation differing somewhat among the various orders of reptiles. In the Crocodilia, Lawton (1937) described the innervation of the alligator adrenal as arising from four to five ganglionic clusters. Fibers from these ganglia enter the gland and associate themselves with medullary tissue. This segmental pattern of innervation was found in both the alligators and caiman examined during this study.

The innervation of the chelonian adrenal is more complex than that of the other reptiles. Because of the close association of the gland with the kidney, it is difficult to determine the exact relationship of the numerous ganglia which are located in or near the adrenal.

In the lizards and snakes, sympathetic ganglia are found chiefly on the dorsal surface of the gland in association with the arterial blood vessels.

The exact nature of the numerous bundles of nerve fibers seen coursing in the capsule and between the parenchymal units is extremely difficult to ascertain from histological preparations. Sudan-stained frozen sections reveal thin sudanophilic sheaths in some of the nerve bundles, which is indicative of the presence of both myelinated preganglionic and unmyelinated postganglionic fibers in the adrenals of reptiles.

The medullary cells receive an abundant supply of unmyelinated fibers which end as dense networks around the cells. Kolossow (1930) observed that the nerve fibers terminate as oval patches on the medullary cells of the turtle, *Emys europaea.* In the alligator lizard, *Gerrhonotus multicarinatus,* Retzlaff (1949) noted that fine nerve fibers terminate on the medullary cells. In the alligator, painted turtle and garter snake, nerve fibers are found running along between the cortical strands and often fine side-branches are given off at right angles, which appear to penetrate into the cortical tissue. The innervation of the cortical cells is a moot question. Some investigators claim that nerve fibers terminate on the cortical cells (Alpert, 1931, in the human; and Retzlaff, 1949, in the alligator lizard). On the other hand, Hollinshead (1936) and Swinyard (1937) found no evidence of cortical tissue innervation in the cat adrenal. Also, Kolossow (1930) was not able to discover nerve endings to cortical cells of the turtle, *Emys europaea.*

The identification of argyrophilic nerve fibers with specific cortical cells is a difficult problem inasmuch as the fibers may not be destined for the cortical cells, but rather for some other nearby tissue such as blood vessels. Swinyard (1937) cautions that the silver techniques employed may possibly stain reticular fibers which may be incorrectly identified as nerve fibers.

Histology

The reptilian adrenal gland is enclosed in a thin connective tissue capsule continuous with the enveloping mesentery or surrounding connective tissue. The capsule is, in general, com-

posed of an outer layer of collagenous fibers continuous with the adjacent connective tissue and an inner layer of reticular fibers continuous with the reticular framework supporting the glandular parenchyma. Elastic fibers are confined to the media of blood vessels that are found in the capsule and trabeculae. The relative amount of collagenous and reticular fibers in the gland varies with both the species and the age of the animal. In young animals, the parenchyma is supported by a thin network of reticular fibers while in old adult animals the reticular framework is supplemented by thick collagenous fibers, the amount of which varies from species to species. In adult turtles, there is considerable infiltration of thick collagenous trabeculae from the capsule, which tend to partition the cortical tissue.

Some species variation is found in the lizards. For example, Retzlaff (1949) observed in *Gerrhonotus* heavy bundles of collagenous fibers surrounding the cortical cells, with fine fibrils originating from these bundles passing among the cells. This pattern is also found in the anguimorphid lizard, *Ophisaurus,* examined in this study, but it is not present in the Scincidae. In the skink, *Eumeces fasciatus,* the cortical cords are enclosed in a network of collagenous fibers; however, fibers do not appear to penetrate among the individual cells comprising a cord.

An interesting and significant departure from the hereinbefore described parenchymal stroma is found in the garter snake. The cortical cords are usually enclosed in a loose connective tissue network composed chiefly of collagenous fibers. This collagenous network is infiltrated with numerous vascular sinuses supported by a fine reticular network. This same arrangement has been reported for the European snake, *Coluber* sp., by Minervini (1904).

The reptilian adrenal gland consists of three kinds of cells—cortical, medullary, and sympathetic nerve cells—arranged in definite patterns which appear to be characteristic for the major phylogenetic groups. In the Crocodilia, the medullary tissue consists of an encircling peripheral zone in addition to small clumps, strands and individual cells scattered in the interspaces and sinuses between the columns of cortical cells. This general crocodilian pattern is found in the alligator and caiman. Reese (1931) and Lawton (1937) have also described this arrangement in the alligator; however, no descriptions of Old World crocodiles were found in the available literature.

A random intermixing of the medullary and cortical components is typical of the Chelonia. The medullary tissue is not concentrated in any particular region of the gland but rather is irregularly distributed among the cortical cell groups as clusters and strands of medullary cells. These clusters are of irregular size and shape, tending to exhibit some species differences. For example, the spotted turtle, *Clemmys guttata,* is unique in having larger and more numerous clumps of medullary cells than those observed in other species of turtles studied. This general chelonian arrangement has been described for several species of turtles: *Testudo tabulata* and *Malaclemys terrapin,* (Vincent, 1896), and *Emys europaea* (Poll, 1906; Radu, 1934). Moreover, a similar random disposition of medullary and cortical components is also present in the avian adrenal (Hartman, et al., 1947).

In the Squamata (lizards and snakes), the medullary tissue is aggravated principally on the dorsal side of the cortical tissue with the degree of intermingling with the cortical tissue varying from species to species. In the horned lizard, *Phrynosoma cornutum,* there is considerable infiltration, while in *Anolis carolinensis* little medullary tissue is found in the cortical parenchyma. This scarcity is also true of the skinks, *Eumeces obsoletus, E. fasciatus* and *Lygosoma laterale.* Among the anguimorphid lizards, Retzlaff (1949) reported that the medullary cells of the alligator lizard, *Gerrhonotus multicarinatus,* are intermingled with the cortical portion. This was also noted in this study for *Gerrhonotus coeruleus* as well as for the related species, *Ophisaurus ventralis.* In addition, Hartman & Brownell (1949) noted some intermingling in the Gila monster, *Heloderma suspectum.*

In the Serpentes, the medullary cells are chiefly aggregated in a compact unit associated closely with arterial blood vessels located on the dorsal side of the capsule. A more or less uniform scattering of small medullary clusters of one to four cells is found in the cortical parenchyma. This arrangement is observed in the garter snake, water snake and other colubrid snakes examined, as well as in the boa constrictor.

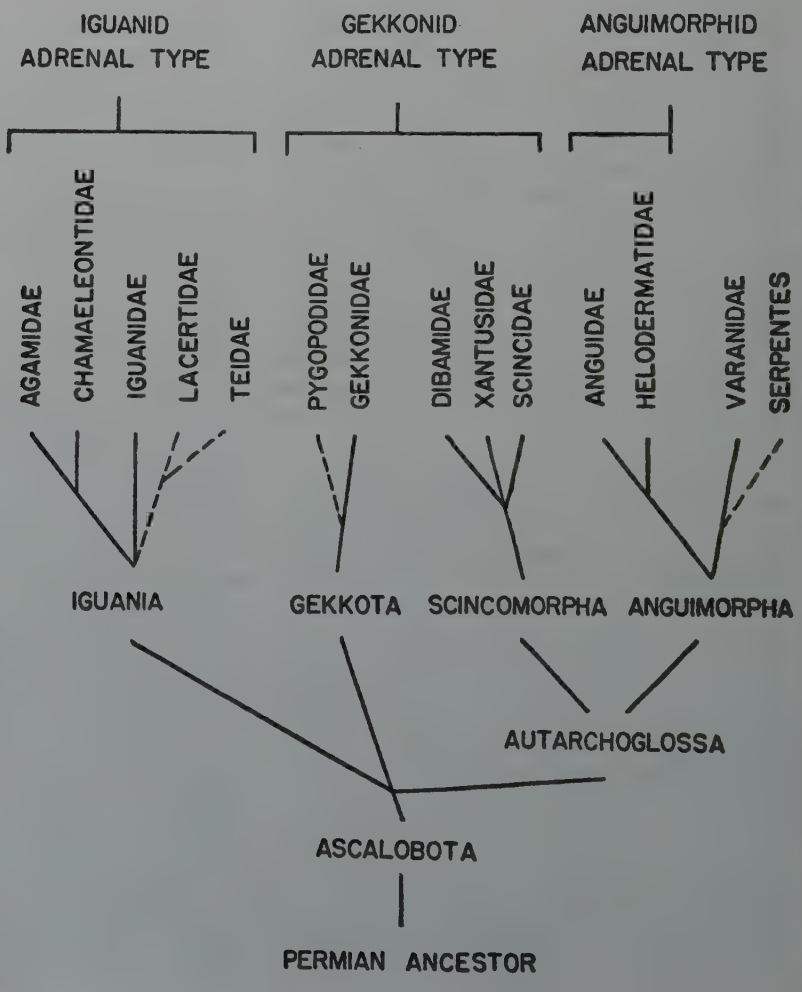
The cortical tissue exhibits considerable variation in shape and arrangement of the cells, with an appearance of a more or less characteristic pattern for each species. In the Crocodilia, namely, the alligator and caiman, the cortical tissue consists of anastomosing cords composed of polyhedral-to-tall columnar cells having a more or less regular arrangement. Probably because of compression, the outer cords in the adrenal often appear to have a more regular cellular arrangement. In this instance, the peripheral cords may consist of a single row of greatly elongated and flattened cells, as noted for the alligator, or as a double row of cells, in the case of the caiman.

In the Chelonia, the cords usually consist of

irregularly-shaped cells which, in longitudinal section, consist of several rows of cells having very indistinct cell outlines and an abundant and irregular distribution of nuclei. The more centrally located columns usually consist of more than two tiers of cells; on the other hand, peripheral cords (as a result of compression) often consist of a single row of greatly elongated and flattened cells. In the family Kinosternidae, the cells tend to have a more regular arrangement, with the cells appearing to be radially disposed in the cords.

The disposition of the cortical cells in the Sauria is of particular interest for the reason that certain phylogenetic trends are apparent. On arranging the various species of lizards (described in this study and elsewhere in the literature) into groups on the basis of the disposition and morphology of the cortical cells, they fall into three general categories having similar histological features: the gekkonid, anguimorphid and iguanid types.

These histological trends appear to substantiate the suggested phylogenetic relationships of the various lizard groups. According to Camp (1923), the Sauria constitute a natural and diversified group arising from some unknown Permian stem reptile which soon divided into two main groups, the Ascalobota and the Autarchoglossa. The former includes the Gekkonidae, Agamidae, Chamaeleontidae and Iguanidae, with the Gekkonidae considered to be the most primitive family. The Autarchoglossa are subdivided into the Scincomorpha, which includes the Xantusidae, Scincidae, Lacertidae and Teidae, and the Anguimorpha, which includes the Anguidae and the platynotid lizards, of which the Varanidae are a part. The Xantusidae are believed to be an intermediate group between the Ascalobota and Autarchoglossa, These suggested relationships are shown in Text-fig. 1, modified after Camp (1923). Only those families are included for which histological data are available for comparison.



TEXT-FIG. 1. Comparison of adrenal histological types with suggested phylogenetic relationships of the lizards. (Phylogenetic chart modified from Camp, 1923).

The characteristic histological feature of the gekkonid type of adrenal is the radial arrangement of tall wedge-shaped columnar cells into cords having a consistent disposition of the nuclei at the periphery of the cords. This arrangement is found in the four species of the Gekkonidae described in this study as well as in the three species belonging to the family Scincidae. The gekkonid pattern is also observed in the Dibamidae and Pygopodidae, and has been described in the Xantusidae by Miller (1952).

The anguimorphid pattern consists of an irregular arrangement of polyhedral low columnar cells with no general tendency to form cords consisting of regularly arranged tiers of cells. This arrangement is found in the glass snake, *Ophisaurus ventralis*, and the closely related species, *Gerrhonotus coeruleus*. Retzlaff (1949) has also described this irregular disposition of cortical cells in the latter genus. Hartman & Brownell (1949) found a similar pattern to exist in another anguimorphid lizard, the Gila monster, *Heloderma suspectum*.

The disposition and morphology of the adrenal cortical cells in the Iguania is somewhat variable, even within family groups; however, all the species that have been described appear to have a more or less regular arrangement of the cells into cords. In the Agamidae, Vincent (1896) describes the cortical tissue of *Uromastix hardwickii* as comprised of cords of cells consisting of a double row of tall columnar cells each having a centrally placed nucleus. Minervini (1904) pictures the cortical cords of the *Chameleon* sp. as composed of small polyhedral cells with centrally located nuclei. In the Iguanidae, the cortical cells of *Anolis carolinensis* and *Sceloporus undulatus* would fit more or less the above description. On the other hand, cortical cells of the horned lizard, *Phrynosoma cornutum*, are more inclined to be of a tall columnar type with the nuclei tending toward a peripheral position.

While certain anatomical similarities suggest that the Lacertidae and Teidae are closely related to the Scincidae (Camp, 1923), the histological features of the adrenal gland of these two families resemble more closely the iguanid type rather than the gekkonid pattern.

According to Camp (1923), the Gekkonidae represent the most primitive group with the Xantusidae being intermediate between the Gekkonidae and the Scincidae. The Lacertidae are considered an offshoot from the Scincidae and the Teidae as a branch of the Lacertidae. The Anguimorpha are believed to have separated from the Scincomorpha very early in the Mesozoic era.

If, as Camp points out, the Gekkonidae represent the most primitive group, then it would seem justifiable to consider that the gekkonid type of adrenal found in the Gekkonidae represents a primitive morphological condition. This type is also present in the following groups: Xantusidae, Scincidae, Dibamidae and Pygopodidae. The Iguania, on the other hand, have diverged from the gekkonid pattern, showing only an occasional similarity as, for example, in *Phrynosoma cornutum*. Furthermore, the Anguimorpha (Anguidae and Helodermatidae) show little similarity to the gekkonid or iguanid types and have in their long geologic separation either undergone an evolutionary modification from the primitive gekkonid pattern or else may have been derived from primitive Ascalobota differing entirely from the primitive Gekkonidae.

The cortical cells of the Serpentes are arranged into cords having a characteristic pattern. In the garter snake, *Thamnophis sirtalis*, the cells are radially arranged into cylindrical cords which exhibit a striking regularity and polarity. In longitudinal sections of a cord, the cells are disposed in two rows of tall columnar cells having a double row of nuclei aligned lengthwise near the center of the cord. In transverse section, a cord consists of a radially arranged group of tall, wedge-shaped cells having a centrally located ring of nuclei. This consistent pattern is also found in the water snake, *Natrix sipedon*, and the rattlesnake, *Crotalus viridis*. In the rubber boa, *Charina bottae*, the cords appear to be made up of several tiers of cells having a more irregular arrangement.

Cytology

(a) Medullary cells

Two types of medullary cells can be distinguished in all of the species of reptiles studied on the basis of their tinctorial characteristics: a "light" type of cell usually light yellow in color and having fine granules; and a yellowish-brown "dark" type having coarse granules. The degree of staining intensity and the relative number of each type varies from order to order and from species to species. The two cell types are best distinguished after fixation in solutions containing chromium salts, such as Zenker's, followed by stains containing acid fuchsin (for example, Masson's). Classical literature often refers to the "chromaffin reaction" as a selective staining of the cytoplasmic granules by the chrome salts; however, Coupland (1953) points out that the yellow-to-brown color is the result of the oxidation of a certain amount of catechol amines into corresponding quinones and other complex oxidation products, such as pigments.

The occurence of different medullary cell types has been reported in mammals (Bänder,

1951; Bourne, 1949); birds (Müller, 1929); reptiles (Radu, 1934; Reese, 1931; Retzlaff, 1949; Miller, 1952); and amphibians (Fustinoni & Porto, 1938).

(b) Cortical cells

The most characteristic feature of the cortical cells is the presence of lipid droplets and granular mitochondria, there being an inverse relationship between the amount of each of these cytoplasmic inclusions present. Histochemical studies indicate that the droplets are a mixture of neutral fats, cholesterol and cholesterol esters, and carbonyl-containing lipids. This description portrays, in general, the fundamental functional unit of the cortical tissue found throughout the various vertebrate classes: shark (Dittus, 1941; Hayes, 1941), lungfish (Holmes, 1950; Gérard, 1951), frog (Radu, 1931), bird (Müller, 1929; Miller & Riddle, 1942; Knouff & Hartman, 1951) and mammal (Greep & Deane, 1949; Nicander, 1952).

The number of lipid droplets may vary slightly from cell to cell; however, there is no tendency toward a zonation similar to that found in the mammals. On the other hand, the distribution of the lipid droplets within the cells varies significantly among the different species of reptiles that were studied, and it is possible to distinguish at least three basically different cytological cell types.

The cortical cells of the garter snake are of special interest for they exhibit a polarization in which the lipid droplets are localized at the free end of the cell in closest contact with the blood stream, while the nucleus is placed near the base of the cell. The granular mitochondria are scattered in the interstices between the lipid droplets. This unique disposition of the lipid droplets, mitochondria and nucleus suggests that an exchange of materials occurs at the peripheral surface adjacent to the blood supply while presecretory synthetic processes occur in the region between the nucleus and the free end of the cell. A similar type of functional polarity has been described for the brown pelican (Knouff & Hartman, 1951).

A functional polarity the reverse of that just mentioned is found in certain species of lizards. In the skink, *Eumeces obsoletus*, the lipid droplets and mitochondria are concentrated near the base of the cells with the nucleus located at the peripheral edge next to the blood stream. This interesting cytological arrangement is also found in other species of skinks, geckos, xantusids, dibamids and pygopodids. A satisfactory explanation for this unique functional adaptation cannot be derived from histological studies alone. Miller (1952) found that the lipid content of the cortical cells of *Xantusia* can be experimentally altered; however, the mechanism as to how secretory products reach the blood stream still remains unknown.

The third functional type has the nucleus centrally located and exhibits an even distribution of lipid droplets and mitochondria throughout the cytoplasm, This disposition is found in the cortical cells of the alligator, painted turtle and green anolis.

Summary

1. The adrenal glands of reptiles exhibit a consistent association with the reproductive organs.

2. The vascular supply consists of both arterial and venous blood with the origin and number of vessels supplying the adrenals varying from species to species. A close association with the efferent renal veins and vena cava is common to all species.

3. The nerve supply to the adrenal is derived from lateral sympathetic ganglia with the extent of innervation differing somewhat among the various orders of reptiles. The medullary component of the gland is innervated by networks of unmyelinated fibers; however, only a few fibers are found in the cortical tissue.

4. The reptilian adrenal gland is enclosed in a thin connective tissue capsule composed of an outer layer of collagenous fibers continuous with the adjacent connective tissue and mesenteries, and an inner layer of reticular fibers continuous with the reticular framework supporting the parenchyma of the gland. The relative amount of collagenous and reticular fibers in the gland varies with both the species and the age of the animal.

5. The reptilian adrenal gland consists of three kinds of cells—cortical, medullary and sympathetic nerve cells—arranged in definite patterns which appear to be characteristic of the major phylogenetic groups.

6. In the Crocodilia, the medullary tissue consists of an encircling peripheral zone in addition to small clumps, strands and individual cells scattered in the cortical parenchyma. A random intermixing of the medullary and cortical components is typical of the Chelonia. In the Sauria and Serpentes, the medullary tissue is aggregated principally on the dorsal side of the cortical tissue, with only a slight intermixing.

7. The cortical tissue exhibits considerable variation in shape and arrangement of the cells with the appearance of a more or less characteristic pattern for each species.

8. The disposition of the cortical cells in the Sauria is of particular interest for the reason that certain phylogenetic trends are apparent.

The Sauria can be divided into three general groups: the gekkonid, anguimorphid and iguanid types, on the basis of similar histological features.

9. Two types of medullary cells can be distinguished in all of the species of reptiles studied on the basis of their tinctorial characteristics.

10. The cortical cells are characterized by the presence of lipid droplets and granular mitochondria, there being an inverse ratio between the amount of each of these present. Histochemical studies indicate that the droplets are a mixture of neutral fats, cholesterol esters and carbonyl-containing lipids. The number of lipid droplets varies from cell to cell; however, there is no tendency toward a zonation.

11. Polarity differences in the distribution of the lipid droplets, mitochondria and nuclei in the cortical cells of the various species studied illustrate fundamental variations in the cytology of cortical cells.

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EXPLANATION OF THE PLATES

Plate I

Fig. 1. Transverse section through alligator adrenal (**a**) showing relationship to kidneys (**k**), gonads (**g**), dorsal aorta (**d**), vena cava (**v**) and sympathetic ganglia (**s**). Bouin's fixation and Masson's stain. ×24.

Fig. 2. Transverse section through adrenal of alligator showing sympathetic nerve fibers in capsule, peripheral zone of "light" medullary cells, strands of "dark" medullary cells extending into cortical tissue and irregular shape and arrangement of light-staining cortical cells. Zenker-formal fixation and Masson's stain. ×130.

Fig. 3. Transverse section through adrenal of alligator showing distribution of reticular network supporting parenchyma of gland. Note heavy reticular fibers running lengthwise with cords and fine network of reticular fibers that encircles the cortical cords. Bouin's fixation and Foot's modification of Hortega's silver carbonate technique. ×240.

Fig. 4. Frozen section of alligator adrenal showing distribution of sudanophilic lipids in the cortical cells. Note absence of stain in medullary cells. Baker's formalin fixation and Sudan black B stain. ×240.

Fig. 5. Transverse section of alligator adrenal showing centrally located nuclei and the uniform distribution of granular mitochondria in the lipid-rich cortical cells.

Note cluster of "dark" medullary cells at upper left and strand of "light" medullary cells at right-hand edge. Zenker-formal fixation, post-chromated for 48 hours and stained with aniline-acid fuchsin and methyl blue. ×690.

PLATE II

FIG. 6. Transverse section through adrenal (**a**) of painted turtle, *Chrysemys picta,* showing relationship to kidneys (**k**), gonads (**g**), dorsal aorta (**d**) and vena cava (**v**). Zenker-formal fixation and Masson's stain. ×24.

FIG. 7. Transverse section through adrenal of painted turtle showing disposition of medullary and cortical tissue. Note irregular arrangement of cortical cells into anastomosing cords. Zenker-formal fixation and Mayer's acid hemalum and eosin. ×230.

FIG. 8. Transverse section through adrenal of painted turtle showing close association of medullary cells and sympathetic ganglion cells. Zenker-formal fixation and Mayer's acid hemalum and eosin. ×230.

FIG. 9. Frozen section of adrenal of painted turtle showing distribution of sudanophilic lipids in cortical tissue. Note single sudanophilic nerve fiber in sympathetic nerve at upper edge of photograph. Baker's formalin fixation and Sudan black B stain. ×240.

FIG. 10. Transverse section of adrenal of painted turtle showing distribution of granular mitochrondria in lipid-rich cortical cells. Note centrally located nuclei in cortical cells and clump of "dark" medullary cells at upper edge of photograph. Zenker-formal fixation, post-chromated for 48 hours and stained with aniline-acid fuchsin and methyl blue. ×690.

PLATE III

FIG. 11. Oblique section through adrenal (**a**) of horned lizard, *Phrynosoma cornutum,* showing association with vas deferens (**v.d.**). Note peripheral zone of darker-staining medullary tissue as well as scattered masses among the cortical cells. Zenker-formal fixation and Masson's stain. ×24.

FIG. 12. Transverse section of adrenal of horned lizard showing sympathetic ganglion cells near artery. Note peripheral zone of "light" medullary cells and clumps of "dark" medullary cells lying between the cortical tissue cords. Note regular arrangement of tall columnar cortical cells. Zenker-formal fixation and Masson's stain. ×230.

FIG. 13. Frozen section of adrenal of horned lizard showing intense sudanophilia of cortical cells. Note negative-staining sympathetic ganglion. Baker's formalin fixation and Sudan black B stain. ×240.

FIG. 14. Transverse section of adrenal of lizard, *Anolis carolinensis,* showing dorsal sympathetic ganglion and medullary tissue. Note close association of cortical tissue with vena cava and cord-like arrangement of cortical cells. Zenker-formal fixation and Mayer's acid hemalum and eosin. ×230.

FIG. 15. Transverse section through adrenal of alligator lizard, *Gerrhonotus coeruleus,* showing pigmented capsule, peripheral zone of medullary cells, peripheral zone of "light-staining" cortical cells and irregular arrangement of cortical cells in the parenchyma of the gland. Bouin's fixation and Masson's stain. ×230.

FIG. 16. Transverse section through adrenal of the glass snake, *Ophisaurus ventralis,* showing aggregation of medullary cells, large sinus opening into ventral vein and the irregular arrangement of the cortical cells. Bouin's fixation and Mayer's acid hemalum and eosin. ×230.

FIG. 17. Transverse section through adrenal of the lizard, *Cnemidophorus tessellatus,* showing dorsal aggregation of medullary cells and irregularly arranged cortical cells. Bouin's fixation and Mayer's acid hemalum and eosin. ×230.

PLATE IV

FIG. 18. Transverse section through adrenal of the skink, *Eumeces obsoletus,* showing typical radial arrangement of the tall columnar cortical cells into anastomosing cords. Note disposition of nuclei at the peripheral edge of the cords. Zenker-formal fixation and Masson's stain. ×230.

FIG. 19. Transverse section of adrenal of skink, *Eumeces fasciatus,* showing peripheral position of nuclei and scarcity of granular mitochondria in lipid-rich cytoplasm of cortical cells. Zenker-formal fixation, post-chromated for 48 hours and stained with aniline-acid fuchsin and methyl blue. ×690.

FIG. 20. Frozen section of adrenal of skink, *Eumeces obsoletus,* showing characteristic localization of sudanophilic lipids in central region of cortical cords and clear peripheral nuclear zone. Baker's formalin fixation and Sudan black B stain. ×240.

FIG. 21. Transverse section through the adrenal of the gecko, *Hemidactlyus turcicus,* showing dorsal medullary tissue and cords of radially arranged tall columnar cortical cells with peripherally located nuclei. Zenker-formal fixation and Masson's stain. ×230.

FIG. 22. Transverse section through adrenal of the gecko, *Tarentola mauretanica,* showing similar disposition of cortical cells as noted for *Hemidactylus* and *Eumeces.* Zenker-formal fixation and Masson's stain. ×230.

FIG. 23. Transverse section through adrenal of skink, *Lygosoma laterale*, showing dorsal medullary tissue and cords of radially arranged tall columnar cortical cells with peripherally located nuclei. Zenker-formal fixation and Masson's stain. ×230.

PLATE V

FIG. 24. Transverse section through the adrenal (**a**) of the garter snake, *Thamnophis sirtalis*, showing relationship to ovary (**o**) and mesovarium (**mes.**). Note ovarian artery (**o.a.**) in dorsal capsular region and intimate association of adrenal with the ventrally located vena cava (**v**). Bouin's fixation and Masson's stain. ×28.

FIG. 25. Transverse section through the adrenal of the garter snake showing large capsular nodule of undifferentiated cortical cells, dorsal aggregation of medullary cells at right of nodule and cords of radially arranged tall columnar cortical cells with nuclei arranged in regular rows at center of cord. Note small groups of dark-staining medullary cells dispersed between the cortical cords and the extreme vascularity of the gland. Bouin's fixation and Masson's stain. ×120.

FIG. 26. Frozen section of garter snake adrenal showing characteristic localization of sudanophilic lipids at periphery of cortical cords and clear nuclear zone in center of cords. Baker's formalin fixation and Sudan black B stain. ×240.

FIG. 27. Frozen section of garter snake adrenal showing distribution of Shultz-positive substances. Note slight staining of undifferentiated cortical cells in capsular connective tissue. Baker's formalin fixation and Shultz cholesterol technique. ×240.

FIG. 28. Transverse section of garter snake adrenal showing characteristic arrangement of tall columnar cortical cells. Note centrally located nuclear zone and uniform distribution of granular mitochondria in lipid-rich cytoplasm. Zenker-formal fixation, post chromated for 48 hours and stained with aniline-acid fuchsin and methyl blue. ×690.

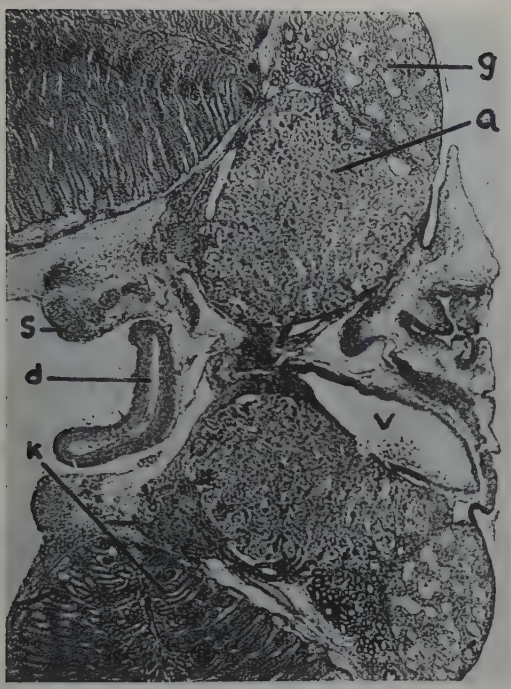

FIG. 1

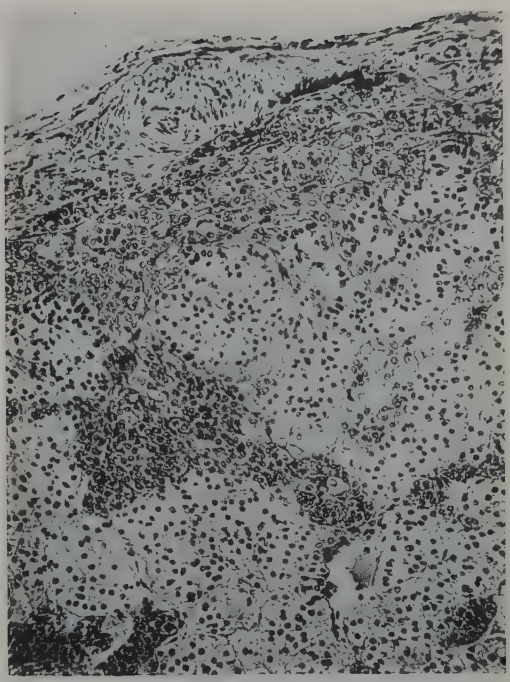

FIG. 2

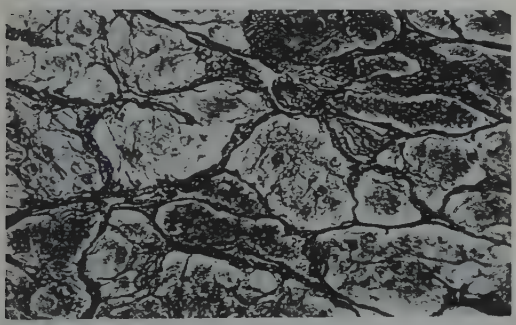

FIG. 3

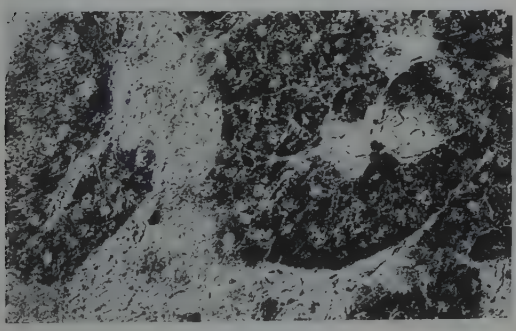

FIG. 4

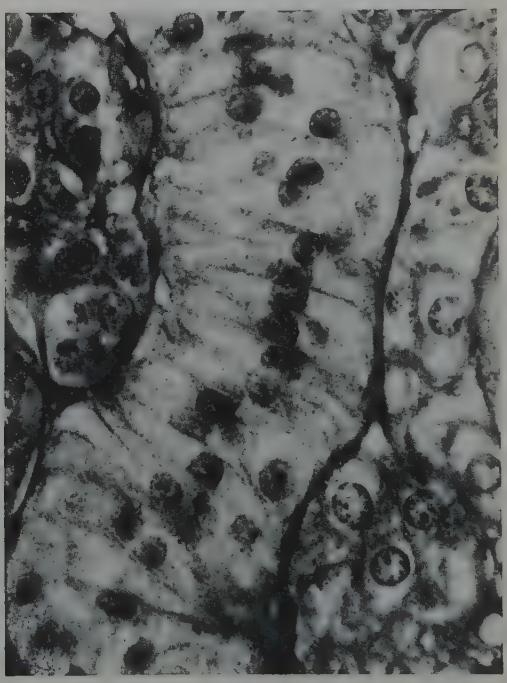

FIG. 5

A COMPARATIVE STUDY OF THE MORPHOLOGY AND HISTOCHEMISTRY
OF THE REPTILIAN ADRENAL GLAND

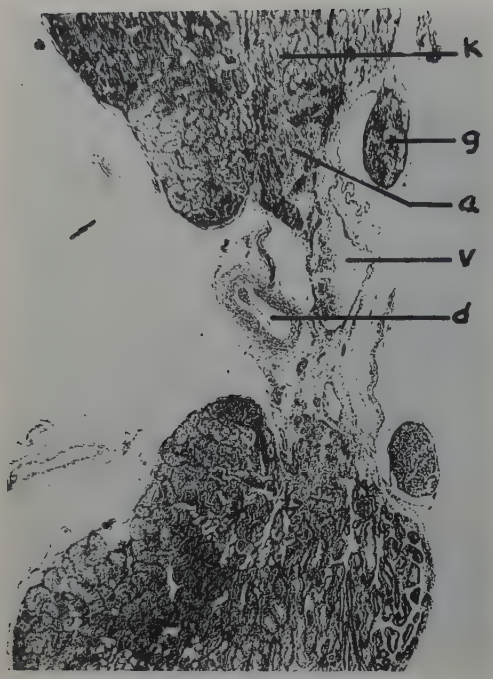

FIG. 6

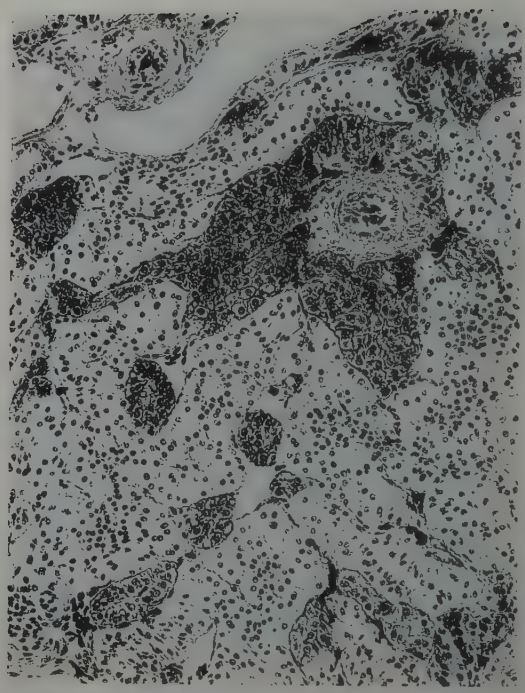

FIG. 7

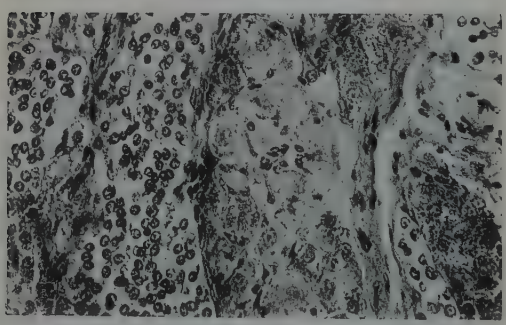

FIG. 8

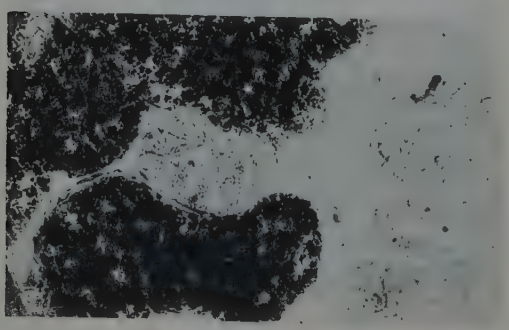

FIG. 9

FIG. 10

A COMPARATIVE STUDY OF THE MORPHOLOGY AND HISTOCHEMISTRY OF THE REPTILIAN ADRENAL GLAND

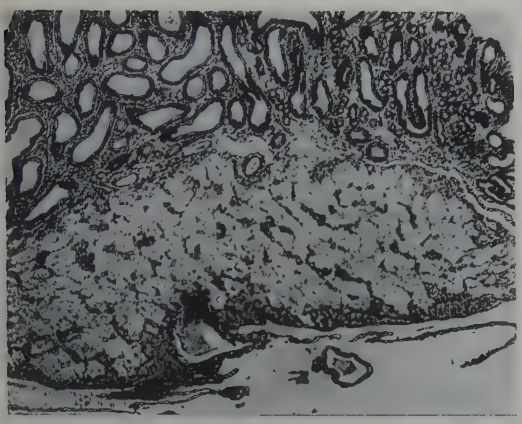

FIG. 11

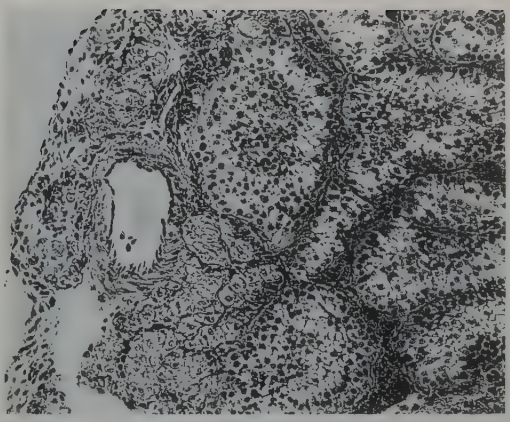

FIG. 12

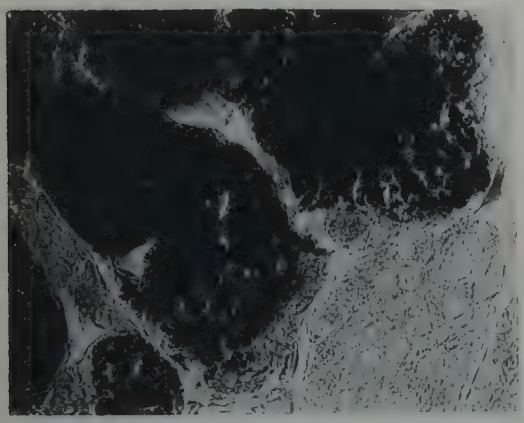

FIG. 13

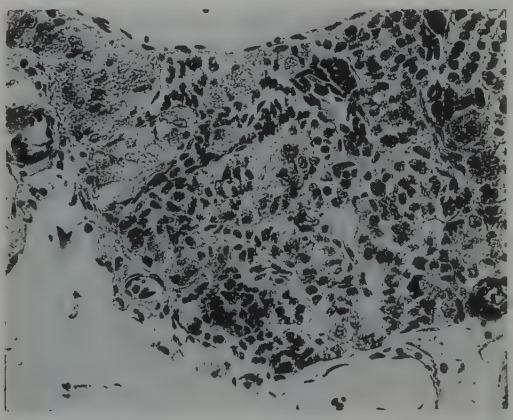

FIG. 14

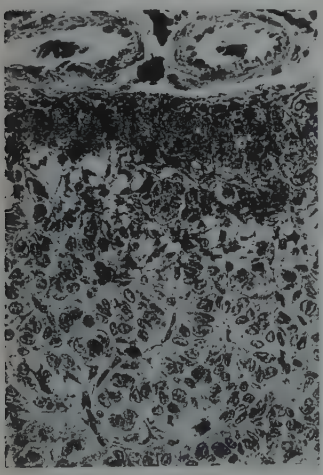

FIG. 15

FIG. 16

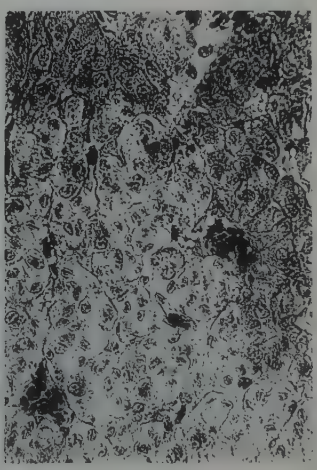

FIG. 17

A COMPARATIVE STUDY OF THE MORPHOLOGY AND HISTOCHEMISTRY
OF THE REPTILIAN ADRENAL GLAND

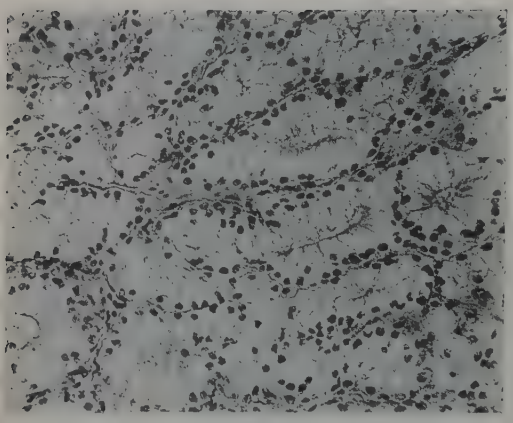

FIG. 18

FIG. 19

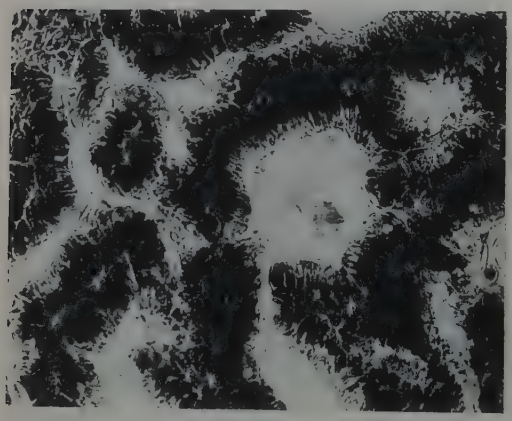

FIG. 20

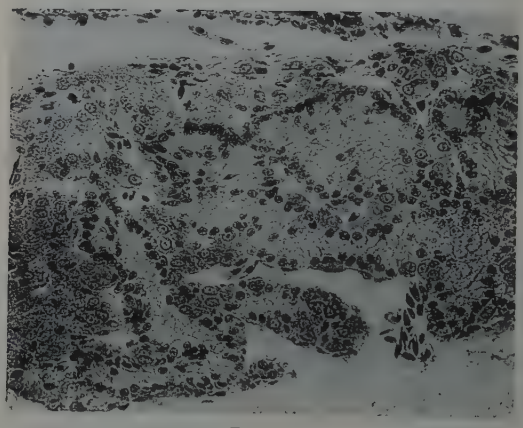

FIG. 21

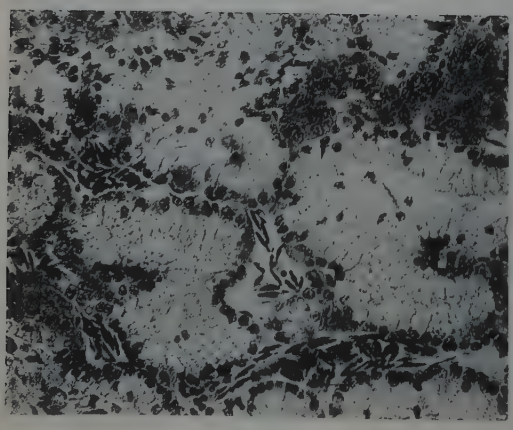

FIG. 22

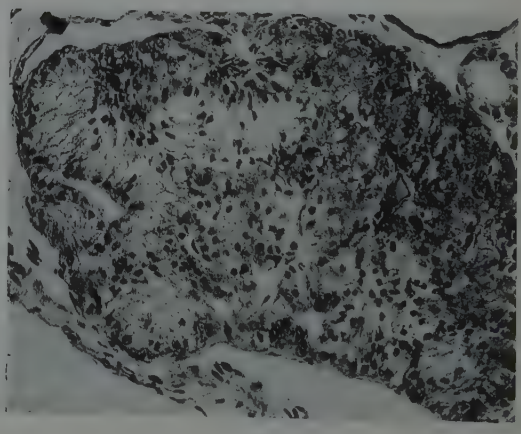

FIG. 23

A COMPARATIVE STUDY OF THE MORPHOLOGY AND HISTOCHEMISTRY
OF THE REPTILIAN ADRENAL GLAND

FIG. 24

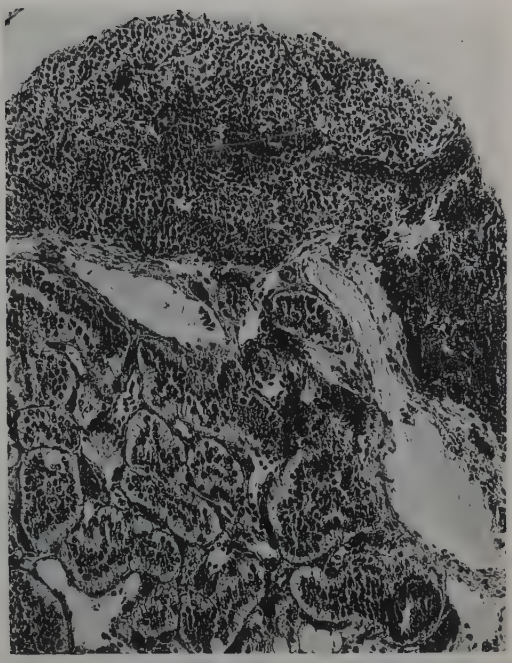

FIG. 25

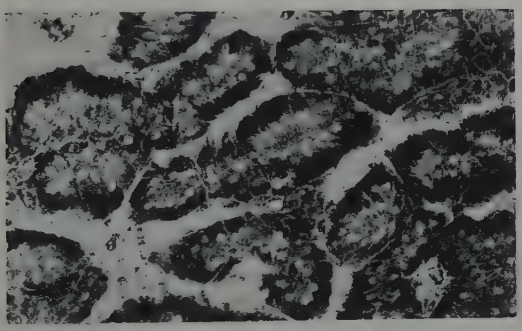

FIG. 26

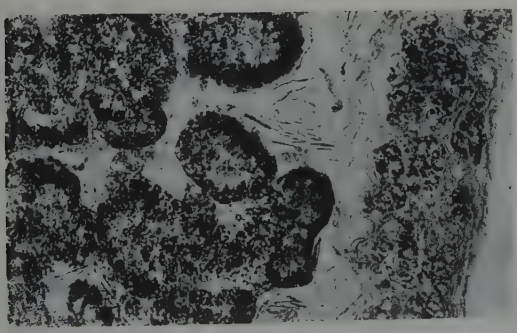

FIG. 27

FIG. 28

A COMPARATIVE STUDY OF THE MORPHOLOGY AND HISTOCHEMISTRY OF THE REPTILIAN ADRENAL GLAND

11

Hormonal Control of the Sexually Dimorphic Pigmentation of *Thalassoma bifasciatum*[1]

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(Plates I-III)

THE bluehead, *Thalassoma bifasciatum* (Bloch), shows the blue-headed condition only in the adult males while immature fish and females show quite other colors and patterns. The fact that the males are sexually mature some time before they develop their strikingly different secondary sex characters has led to a considerable taxonomic confusion. The females and young have gone under the name of *T. nitida* (Günther) and *T. nitidissima* (Goode), depending on the color phase they showed. The males have been referred to as *T. subfurcatus* Nichols. This situation was recognized by Longley (1914, 1915) but not generally accepted by ichthyologists. As recently as 1930 Nichols still considered the possibility of them being separate species. Breder (1927) and Beebe & Tee-Van (1928) were disposed to the view that they were all one species. Tee-Van (1932) referred *T. nitidum* and *T. subfurcatus* Nichols to the synonomy of *T. bifasciatum*. *T. nitidissima* was added to the synonomy by Beebe & Tee-Van (1933).

The coloration and pattern of the juvenile, female and young male *Thalassoma* is principally that of a yellow-backed fish with or without a dark lateral stripe. This stripe may be broken up into blocks. Changes in color patterns are observable in the living individuals. Variations of this type of pattern are not the concern of the present study, however.

The presence of males with two different colorations in a single species suggested that the factor instituting the change from small yellow male to larger blue male was hormonal rather than chromosomal. If this were the mechanism, injections of sufficient androgenic hormone given to yellow-phase fish should cause the blue phase to replace it and would clearly establish these two forms as individuals of the same species, accounting for the presence of two types of males and only one type of female.

Darby (1935-1936) reported Testosteron (Ciba) effective in causing " . . . the immature bluehead (*Thalassophryne sp.*)" [*sic*] to take on mature coloration. The designation of the fish used as *Thalassophryne* sp. is probably incorrect as this form is a genus of toadfishes found in the Pacific. There is one report (Günther, 1861) of a single Atlantic species, *Thalassophryne maculosa* Günther, from Panama, but this report is in question. There have been no reports of *Thalassophryne* sp. in the vicinity of the Dry Tortugas. *Thalassoma* was probably the "bluehead" used. No subsequent publication on the problem has been found.

I should like to express my appreciation to Dr. C. M. Breder, Jr., and Miss Priscilla Rasquin for their suggestions and advice during the course of this study.

MATERIALS AND METHODS

The blueheads used in this investigation were collected from tidal flat *Thallasia* beds and around small coral growths in Bimini, Bahamas, B. W. I. Experimental work was done in the Lerner Marine Laboratory at Bimini. While under observation, the fish were kept in aquaria with running sea water. Conch shells were provided for shelter.

Four groups of fish were used. Group 1 comprised "nitidum" and "bifasciatum" fish collected in February and March. "Nitidum" and "bifasciatum" designate color phases and are described in a separate section. These fish were used for study of normal tissue. Group 2 consisted of "nitidum" fish used in a preliminary experiment to determine dosage and medium for suspension of the hormone. Methyl testosterone

[1]This work has been supported in part by a grant from the National Science Foundation.

was dissolved in 95% alcohol and suspended in physiological saline. Injections were given intraperitoneally without anesthesia. Dosage was not equal for all fish, as the saline caused a partial precipitation of the hormone and additional 95% alcohol was added to restore the suspension. The alcohol was found to be toxic and fatalities were numerous. Another set of fish was designated as Group 3. In this case methyl testosterone was dissolved in 95% alcohol, suspended in sesame oil and incubated at 37° C. to evaporate the alcohol. A 1% solution of ethyl urethane dissolved in sea water was used as an anesthetic. A group of fish was collected in June for comparison with Group 1. These fish were designated as Group 4. The ratio of males to females in June differed from the ratio in the February-March period. In March, 28 fish were collected of which nine were males and 19 females. Two to three months later, 18 fish were collected and ten were males, seven females and one a true hermaphrodite having one lobe of testicular tissue and one of ovarian tissue (Pl. III, Figs. 15 & 16).

The numbers of fish, dosages and descriptions of fish in all groups are given in Table 1 with dates of collection, treatment and sacrifice.

All fish were killed by a lethal dose of ethyl urethane. This caused the dispersion of melanin granules, making the fish assume their darkest coloration. Photographs in Pl. I were taken after the fish had been killed, in order to show them uniformly in this darkest and most marked pattern.

Bouin's solution was used for fixation. The gonads were dissected from all fish, imbedded in paraffin and sectioned at 7μ. Sections were stained with Harris's hematoxylin and eosin and with Masson's trichrome stain for connective tissue.

Observations

Color and Pattern.—The predominant color of the "nitidum" stage is yellow. A dark median lateral stripe marks the fish from the snout to the caudal fin, running through the eye. The stripe is sometimes modified into six rectangular areas separated by gray or yellow bars. These intermediate markings are the basis for the distinction between "nitidum" and "nitidissimum," the former notably displaying the gray and the latter the yellow. In "nitidum," the dorsal fin is bordered by light blue and the caudal fin has a dark margin. In shape, the caudal fin is slightly concave as the median rays are shorter than the peripheral rays. Ventral and anal fins are transparent but the pectoral fins have slightly dusky tips. A fish in the "nitidum" stage is shown in Pl. I, Fig. 1.

"Nitidissimum" would appear to be a modification of "nitidum" in which bands of yellow replace the gray ones. This stage is more apt to be a product of activity and environment than of age or sex. Longley & Hildebrand noted that this phase was shown most frequently by resting fish. For the purposes of this study, "nitidissimum" fish are included in the "nitidum" classification.

Blue is the principal color of the "bifasciatum" stage. Longley & Hildebrand described a blue or violet coloration on the head and the throat extending to the base of the dorsal fin. Fish in the "bifasciatum" phase kept in the aquaria seldom showed the violet coloration but the blue was vivid. The body of the fish is banded by two black bars situated behind the pectoral fins. The black bars are separated by a blue bar. The remainder of the body of the fish from the second black bar is greenish. As in the case of the violet color, green is seldom observed to be displayed by fish in aquaria; blue or yellow is the color shown. The pectoral fins have distinct black tips. The caudal fin is markedly forked. The black bars of the body extend through the dorsal fin which is otherwise transparent. Fish in this phase are larger than "nitidum" phase fish. Pl. I, Fig. 4, pictures an untreated "bifasciatum" fish.

The injection of methyl testosterone produced "bifasciatum" coloration and pattern in "nitidum" phase fish regardless of sex. Pl. I, Fig. 2, shows a treated male; Fig. 3 shows a treated female. The first sign of the "bifasciatum" coloration became apparent four days after injection. During these four days the yellow color and "nitidum" pattern gradually faded. The blue coloration started in the head region and three days later the black bars in the region of the pectoral fins began to appear. The initial black of the bars originated at the point where the bar would have bisected the stripe of the "nitidum" phase. The tips of the pectoral fins which cover this region became darker because of an increase in the number of melanophores. Pl. II, Figs. 5 and 6 show the difference in the amount of pectoral fin pigmentation between a "nitidum" stage and an experimentally-induced "bifasciatum" stage. In the ten days following the appearance of the black bar, the blue coloration spread over the entire body of the fish, gradually increasing in intensity.

In the aquaria, the fish were light blue and the "bifasciatum" pattern was evident but not intense. When sacrificed in urethane with the resulting full expansion of the pigment cells, the fish were vivid blue and the pattern well defined in all but two of the 15 fish. These two fish were blue but showed both faint black bars and faint black stripes. The experimentally-induced blue color, however, was never as intense as the blue

TABLE 1. SUMMARY OF FOUR GROUPS OF *Thalassoma bifasciatum*.

	Date collected	Phase	Number	Average standard length in mm. at time of sacrifice	Average total length in mm. at time of sacrifice	Treatment Date–Type	Date of sacrifice	Gonads
Group 1	March 4	bifasciatum	12	84	103	None	March 4	Testes 12
	Feb. 19	nitidum	9	65	75	None	Feb. 19	Testes 4 Ovaries 5
Group 2	Feb. 18	nitidum	16			Feb. 18, 1 mg. methyl testosterone		
			15			Feb. 25, 1 mg. methyl testosterone		
			3	64	74	March 8, 2 mg. methyl testosterone	March 26	Ovotestes 3
	Feb. 25	nitidum	6	59	69	Feb. 25, 0.1 cc. 95% alcohol	March 26	Testes 2 Ovaries 1
Group 3	March 6	nitidum	16	63	72	March 6, 0.5 cc. sesame oil	March 26	Testes 2 Ovaries 14
		nitidum	15	61	71	March 6, 2 mg. methyl testosterone	March 26	Testes 2 Ovotestes 13
Group 4	Early June	bifasciatum	6	78	94	None	Same day as collected	Testes 6
		nitidum	18	60	70	None		Testes 10 Ovaries 7 Hermaph-rodite 1

displayed by the wild fish in the "bifasciatum" phase. Comparison of the wild and the experimental "bifasciatum" coloration showed that the green element was lacking in the experimentals.

Goodrich & Biesinger (1953), working on the histology of the coloration of *Thallasoma bifasciatum,* noted that the underlying tissue of the green scales had a layer of guanophores as well as the xanthophores and melanophores present in the black and the blue areas. The number of melanophores present in the green areas was less than the number present for the other two colors: 200 per sq. mm. in green areas and 450-500 per sq. mm. in black areas. However, the number of xanthophores present in the green areas was greater than that present in black or blue areas: 350 per sq. mm. for green, 15 scattered per sq. mm. for blue and 45 per sq. mm. for black. All the numbers given were approximations.

There are two possible explanations for the nonappearance of the green color: (1), that the two weeks' time the experimental fish were maintained after injection was not long enough to build up the concentration of xanthophores and guanophores necessary to produce green coloration; and (2), that an improper diet caused loss of color in the xanthophores (Fox, 1953). The one fish from Group 2 which was maintained for more than six weeks did show some green coloration when anesthetized in ethyl urethane.

Histology.—In histological section, distinction could be made between normal "nitidum" testes and normal "bifasciatum" testes. The testes of males in the "nitidum" phase contained large reservoirs of mature sperm aggregated in the lumen of the sperm duct and held in the tubules. The bulk of the testes was comprised of large cysts with thin membranes containing mature sperm. Very few early or intermediate stages of spermatogenesis could be noted (Pl. II, Fig. 7).

Small reservoirs of mature sperm were seen in the tubules of "bifasciatum" testes. The greater part of the testes, however, was made up of cysts in early or intermediate stages of spermatogenesis. A few cysts were present which contained mature sperm and these appeared to be in the stage immediately preceding rupture. The sperm cells were clustered on or near the walls of the cysts while the centers of the cysts were empty (Pl. II, Fig. 8). The testes of fish in this stage were half as large as testes from fish in the "nitidum" phase. No interstitial cells like those described by Courrier (1921) were identified. The specific source of the androgenic hormone responsible for the blue color and pattern is as yet unknown.

The presence of large quantities of sperm in the testes of "nitidum" males indicates that sexual maturity for the males is attained in this phase. The absence of large sperm reservoirs in "bifasciatum" fish suggests that some spawning has occurred during the "nitidum" phase to deplete the sperm reservoirs.

Normal ovaries showed early and intermediate maturation stages as well as mature eggs. The follicles were large and the ova were surrounded by heavy chorionic membranes and encircled by follicular cells (Pl. II, Fig. 9).

Sesame oil injections used for control purposes produced variations from the normal in both "nitidum" males and females. The testes of the two males of the group showed all mature sperm, indicating that spermatogenesis had been accelerated. The sperm were held within the cysts and the membranes were well-defined and thick. Only a few small sperm reservoirs could be seen. Spaces formerly occupied by the sesame oil which had been dissolved by the histological procedure were scattered throughout the gonads (Pl. II, Fig. 10). The size of the testis was not affected by the sesame oil.

Ovaries from sesame oil-injected females were about one-quarter the size of the normal ovaries. Each ovary was sac-like in structure and enclosed a large empty lumen. The walls of the sac were as thin as three to four cell layers in some fish. No mature eggs were seen; however, loose follicular cells were present. Very little connective tissue was seen. A few chorionic membranes were observed, which suggested that some eggs had been resorbed (Pl. III, Figs. 11 & 12).

The testes of males injected with methyl testosterone showed only mature sperm. The membranes of the cysts were thin and there were large reservoirs of mature sperm (Pl. III, Fig. 13). The androgenic hormone accelerated the production of mature sperm. Early and intermediate stages of maturation were absent.

The gonads of the 13 other fish which had received injections of methyl testosterone showed ovarian tissue interspersed with maturation stages of spermatogenesis (Pl. III, Fig. 14). The predominance of ovarian tissue indicates that these fish were females before injection. The ovaries were drastically reduced in size to less than a quarter the size of ovaries of normal females collected at the same time and location. The ovary appeared as a sac-like structure, hollow and collapsed. In section, maturation stages, mature eggs and degenerating eggs could be seen. Collapsed chorionic membranes were plentiful and there was an abundance of connective tissue. All stages of spermatogenesis could be identified in the ovaries and small res-

ervoirs of mature sperm were present. The testicular tissue was present throughout the organ and did not appear to be especially abundant in any specific part. This tissue is thought to have arisen from primordial germ cells.

Discussion

Previous experiments with sex reversal in fishes have been carried out primarily on representatives of the poeciliid fishes. Particular emphasis has been given to *Xiphophorus helleri* (Heckel). The development in the female of the secondary sex characters of the male of this species has served as an external index of possible sex reversal.

Essenberg (1926) reported spontaneous sex reversal from female to male in *X. helleri.* The reversal occurred even after the female had given birth to one or more broods. After reversal the secondary sex characters of the male were exhibited by the fish but the female shape was retained. On the basis of these observations, Essenberg concluded that sex in this species was determined and controlled hormonally and not genetically and stated that any agent or condition which tends to decrease the capacity of the female sex hormone secretion beyond a certain limit becomes an immediate factor in sex reversal in the female.

In 1937, Witschi & Crown found that nonpregnant female *X. helleri* subjected to testosterone propionate (Ciba) dissolved in the aquarium water, absorbed their eggs and the ovaries resembled testes although no spermatogenesis was observed. The secondary sex characters of the male were displayed. Pregnant females under similar treatment aborted or absorbed their eggs within one to two days.

Baldwin & Goldin (1939) reported histological changes in ovaries of 50% of a group of virgin female *X. helleri* injected with testosterone propionate dissolved in sesame oil. The changes included absorption of the gonad and the presence of some phases of spermatogenesis. Baldwin & Li (1942) demonstrated the possibility of complete sex reversal in adult female *X. helleri* treated with gonadotrophic (human chorionic) hormone, and later (1945) cited two cases of ovotestes in males that had been injected with alpha-estradiol benzoate.

Burger (1942) treated male *Fundulus* with testosterone propionate and found that it had only a slight stimulating effect on the male germ cells. An increased coloration was noted and an increase in the extent of the testicular duct system.

Mature female *Gambusia holbrooki* Girard developed masculinized anal fans when treated with testosterone propionate. Immature females showed inhibition of growth (Hamon, 1946). Female *Gambusia affinis* maintained in a solution of ethynyl testosterone for two to four days developed masculine secondary sex characters and retained these characters up to 60 days after they had been replaced in fresh water. On the basis of this experiment, Turner (1946) stated that this response was a clear indication that the genetic factors for the male characters were present in the female but normally did not develop because of insufficient androgenic hormone.

Zei (1950) described typical sex reversal in three Mediterranean fishes, *Maena smaris* (Linnaeus), *M. chryselis* (Cuvier & Valenciennes) and *Pagellus erythrinus* (Cuvier & Valenciennes). The fish are female for the first half of their lives and male the second. Transformation takes place at the 13-15 cm. size. The males may be identified by brighter color, larger size and better developed anal and dorsal fins.

The histology of hermaphroditism in *Serranus* and *Sargus* was described by Van Oordt (1929). Lavenda (1949) reported the presence of developing testicular tissue in functional female sea bass, *Centropristes striatus.* The testicular tissue arose from the epithelial lining of the oviduct.

According to D'Ancona (1950) hermaphroditism in teleosts is found only in some species of *Sparida* and *Serranida.* In the case of the Sparidae, he assumes two different germinal areas for gonad origination reaching maturity successively and each producing distinct sex differentiators known as *gynogenine* and *androgenine.*

Courrier (1921) described interstitial cells in the testes of representatives of the Gobiidae, Callionymidae, Cottidae, Cichlidae and Gasterosteidae. The function of control of the secondary sex characters was ascribed to the interstitial cells. Van Oordt (1925) found no interstitial cells in the testis of *X. helleri* (Heckel). Craig-Bennett (1931), studying *Gasterosteus aculeatus* Linnaeus, found interstitial cells most abundant during the breeding season and inconspicuous in quiescence.

The existence of teleost androgens has been experimentally demonstrated. In 1937, Hazleton & Goodrich accelerated comb growth of two capons with an extract of the testes of salmon, *Oncorhynchus kisutch.* Potter & Hoar (1954) reported androgens in the testes of *Oncorhynchus keta* Walbaum. Histological examination of the testes showed that interstitial cells and extracts produced comb growth in baby chicks. The number of interstitial cells was dependent on the season, as with *Gasterosteus aculeatus* Linnaeus.

Results of the present report show that *T. bifasciatum* is not a hermaphroditic fish but is possibly a progynous species in which all individuals start life as female and later become male. Development of the "bifasciatum" phase is under the control of the androgenic hormone as injection of male hormone brought about the male coloration regardless of the sex of the "nitidum" fish used. However, the appearance of the blue, "bifasciatum" coloration is not concomitant with sexual maturity, because mature testes are found in the yellow phase, but it may be a function of age and length of time the androgenic hormone has been in action.

The sac-like structure and reduced size of the ovary of the hormone-treated fish may be the result of the sesame oil in which the hormone was suspended. The ovaries of females treated with sesame oil alone showed a similar modification in size and structure. It is of interest to note that the sesame oil was inert in regard to testicular tissue and caused no change in size or structure of testes.

Summary

1. All *Thalassoma bifasciatum* with blue heads are males but fish with largely yellow coloration are male, female or juvenile.

2. Large reservoirs of mature sperm were found in testes of yellow males, indicating that sexual maturity is reached in this coloration phase. The testes of blue males contained numerous early and intermediate stages of spermatogenesis but very few mature sperm. The testes of yellow males were twice as large as the testes of blue males.

3. Intraperitoneal injection of methyl testosterone in yellow-phase fish produced blue-phase color and pattern in both sexes.

4. Androgenic hormone is responsible for the change from yellow to blue phase. The first indication of color change was noted on the head region four days after injection, the first indication of pattern change was observed three days later, on and under the tips of the pectoral fins with an increase in the number of melanophores present.

5. Methyl testosterone produced the development of testicular tissue and the regression and absorption of ovarian tissue in yellow females and acceleration in the production of sperm in yellow males.

6. Testicular tissue that developed in yellow females evidently arose from the primordial germ cells.

7. The experimentally produced ovotestes contained large amounts of connective tissue absent in the gonads of both normal females and males.

8. No interstitial cells for androgenic hormone elaboration could be found in testes from blue-phase fish.

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EXPLANATION OF THE PLATES

Plate I

Fig. 1. Female bluehead in the "nitidum" phase. × .71.

Fig. 2. "Nitidum"-phase male three weeks after injection of 2 mg. methyl testosterone. The black lateral stripe has been obliterated and the color and pattern of the "bifasciatum" stage has begun to appear. × .74.

Fig. 3. Female "nitidum"-phase fish after injection of 4 mg. methyl testosterone, showing pronounced "bifasciatum" color and pattern. The blue color is indistinguishable in black and white reproduction. × .71.

Fig. 4. Untreated male in "bifasciatum" phase. × .84.

Plate II

Fig. 5. Tip of pectoral fin of a "nitidum"-phase fish. × 100.

Fig. 6. Tip of pectoral fin from a fish in an experimentally-produced "bifasciatum" phase. × 100.

Fig. 7. Testis of untreated "nitidum"-phase fish showing relatively large numbers of mature sperm and a few maturation stages. × 600.

Fig. 8. Testis of untreated "bifasciatum"-phase fish showing predominance of early stages of spermatogenesis and relatively few mature sperm. × 600.

Fig. 9. Ovary of untreated "nitidum" fish. × 90.

Fig. 10. Testis of "nitidum"-phase fish three weeks after injection of 0.5 cc. sesame oil. × 100.

Plate III

Fig. 11. Ovary three weeks after injection of 0.5 cc. sesame oil, showing decrease in size of the organ and decrease in size and number of eggs. × 100.

Fig. 12. Detail of Figure 11, showing collapsed chorionic membrane. × 600.

Fig. 13. Testis after injection of 2 mg. methyl testosterone, showing only mature sperm and no stages of early spermatogenesis. × 600.

Fig. 14. Gonad, designated as an ovotestis, three weeks after injection of 2 mg. methyl testosterone. Note early stages of spermatogenesis and degenerating ova. × 600.

Fig. 15. One lobe of gonad of hermaphroditic "nitidum" fish, showing only testicular tissue. × 110.

Fig. 16. The other lobe of the gonad pictured in Figure 15, showing typical ovarian structure. × 110.

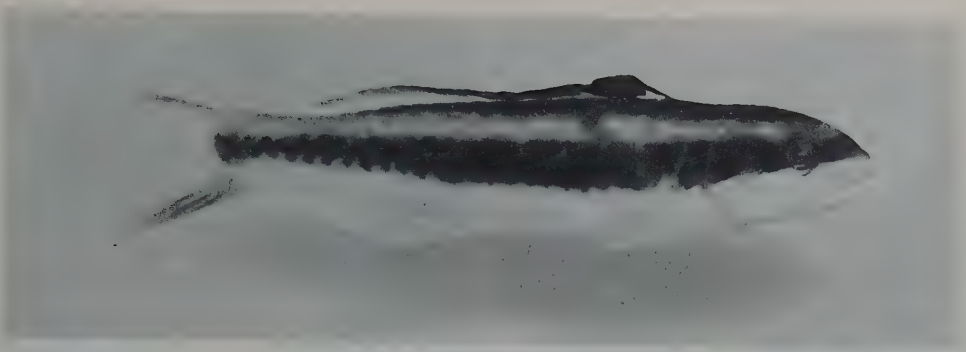


FIG. 1

FIG. 2

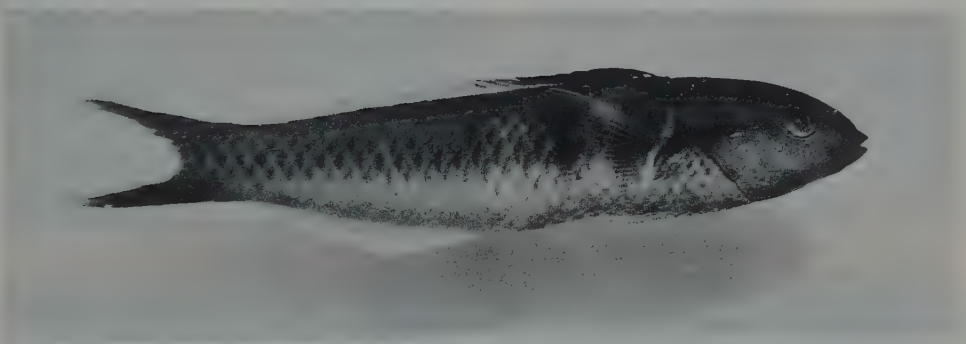


FIG. 3

FIG. 4

HORMONAL CONTROL OF THE SEXUALLY DIMORPHIC
PIGMENTATION OF THALASSOMA BIFASCIATUM

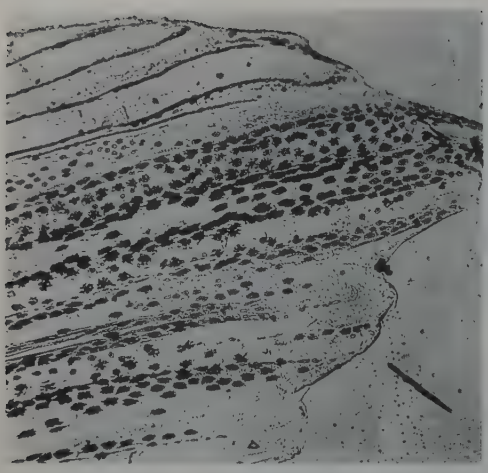


FIG. 5

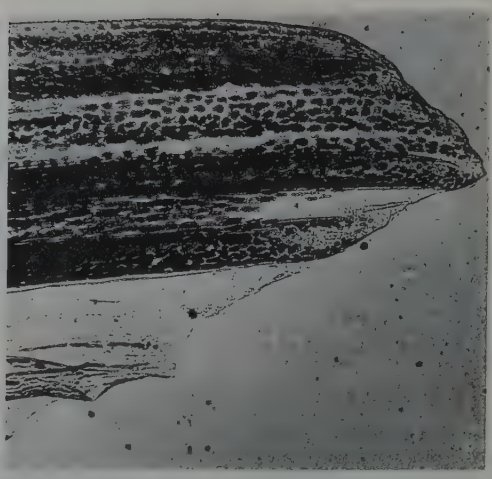


FIG. 6

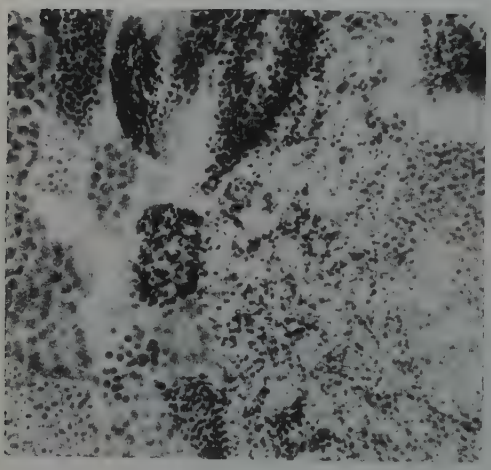


FIG. 7

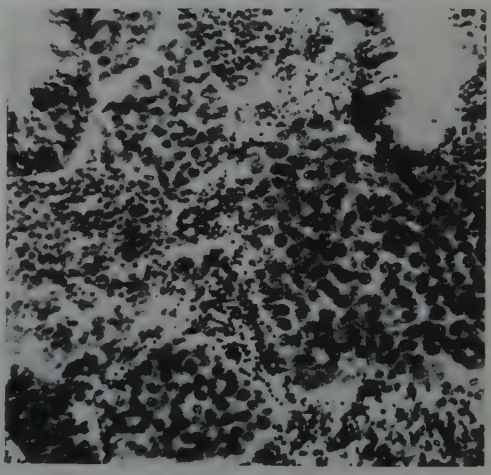


FIG. 8

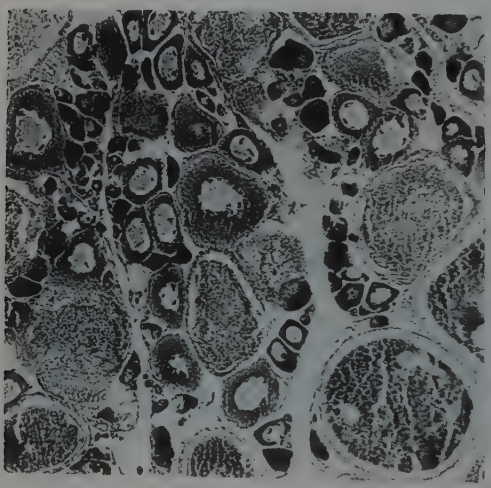


FIG. 9

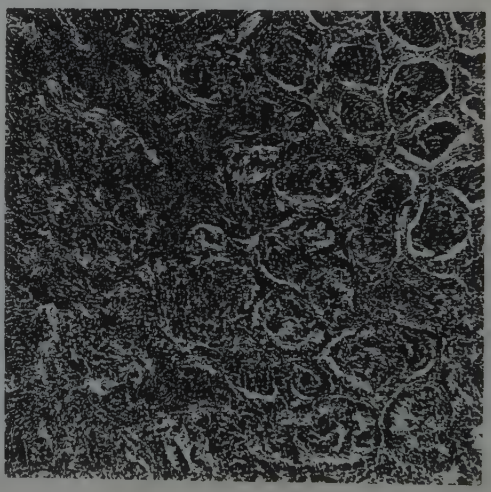


FIG. 10

HORMONAL CONTROL OF THE SEXUALLY DIMORPHIC PIGMENTATION OF THALASSOMA BIFASCIATUM

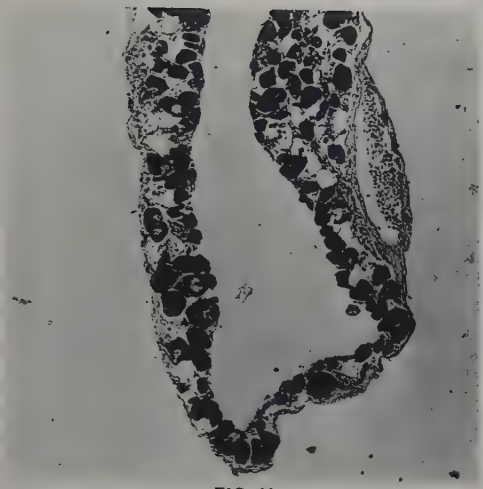


FIG. 11

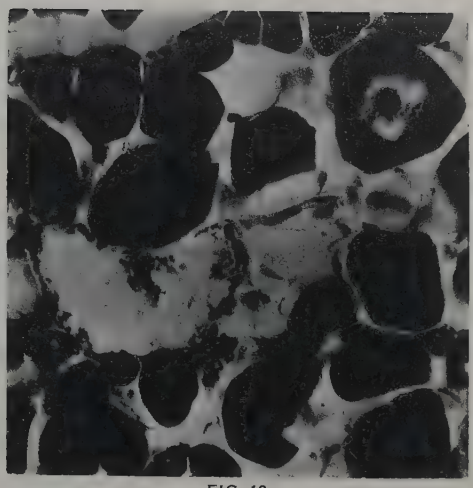


FIG. 12

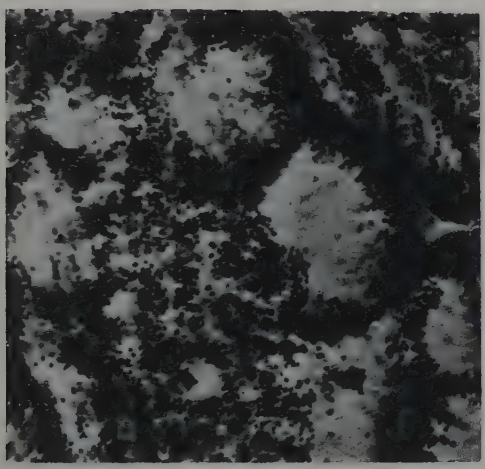


FIG. 13

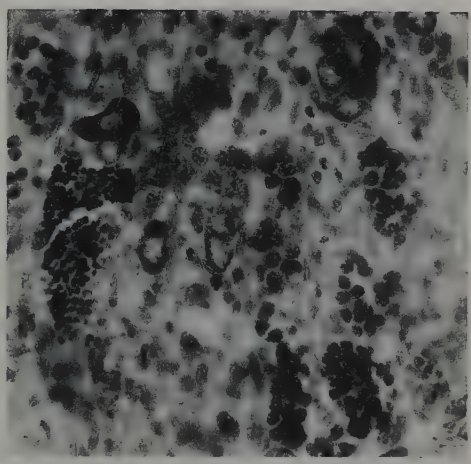


FIG. 14

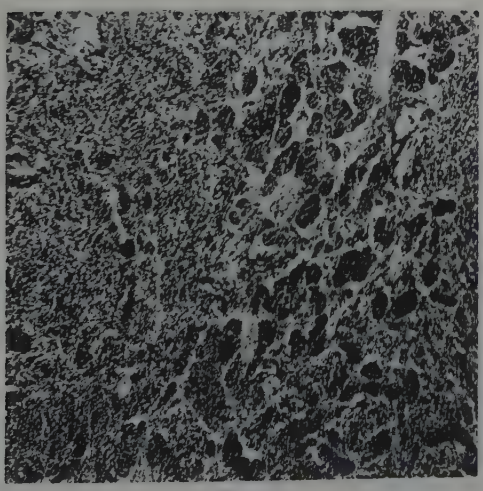


FIG. 15

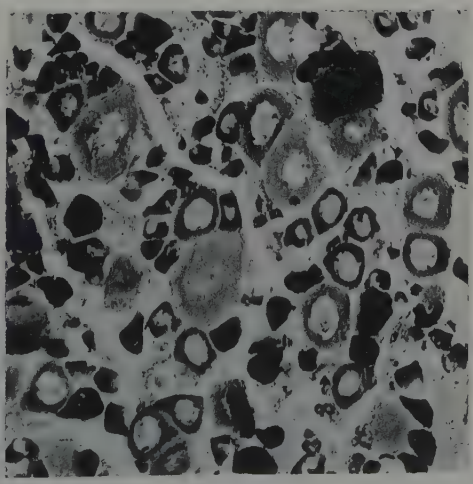


FIG. 16

HORMONAL CONTROL OF THE SEXUALLY DIMORPHIC PIGMENTATION OF THALASSOMA BIFASCIATUM

12

The Use of Copper Sulfate as a Cure for Fish Diseases Caused by Parasitic Dinoflagellates of the Genus *Oodinium*

Robert P. Dempster

Steinhart Aquarium, California Academy of Sciences, San Francisco

(Plate I; Text-figure 1)

Introduction

CORAL-REEF fishes from the Hawaiian Islands have been received by the Steinhart Aquarium for many years and until 1951 they were relatively free from any disease that could be considered as epidemic. In that year, however, about ten days after an exceptionally large shipment arrived from Honolulu, a gill disease broke out in the tanks. At first the fishes were not suspected of having any specific disease. Many of them congregated near the surface and were observed to be respiring very rapidly, obviously in great distress. It was apparent that either the water was deficient in oxygen or the fishes for some reason were not able to utilize the oxygen that was present. On the assumption that the tanks had become contaminated, they were drained and refilled with fresh sea water and the circulation of water was substantially increased, but the fish were not relieved of their respiratory trouble. As time went by, more individuals congregated near the surface, gasping for air, and it was not long before some of them died.

Microscopic examination revealed innumerable minute, oval, parasitic organisms clinging to the gill filaments of the dead fish (Plate I), so thickly planted that they were obviously interfering with the respiratory function of the gills. They were determined to be a species of *Oodinium,* possibly *ocellatum.* Left overnight in a dish of water, they were re-examined the next morning and it was found that some had divided once and many twice, so that all had passed into the 2- or 4-cell stage of development within the period of about 24 hours. Nigrelli (1936) found that each *Oodinium* organism, after becoming detached from the gills of a fish, settled to the substratum, where it gave rise to palmella stages of 2, 4, 8, 16, 32, 64 and 128 cells. One more palmella division took place to form 256 flagellated, free-swimming dinospores. Later the dinospores settled to the bottom, where they developed into typical peridinian dinoflagellates, the infective form. These dinoflagellates, which are also free swimming, apparently invade the branchial chamber of the fish, become attached to the gill filaments and metamorphose into the parasitic form.

According to Jacobs (1946), *Oodinium ocellatum* is the first dinoflagellate known to parasitize marine vertebrates.

Treatment of Marine *Oodinium* Infestations

The immediate problem was to determine how to relieve the fish of this very prolific parasite and how to eradicate it from the Aquarium's water system. Several methods of treatment were tried; the most effective entailed the use of copper sulfate. That copper is highly toxic to fish and that it must be used with extreme caution is well known. After making a considerable number of tests with tropical marine fishes, I have found that 0.5 p.p.m. is a safe concentration and is lethal to *Oodinium.* When treating large volumes of water, especially in an aquarium where the amount of untreated incoming water may fluctuate greatly, it may be difficult to maintain the concentration exactly at that level. Excellent results can be achieved even though the copper concentration is allowed to fluctuate from 0.4 p.p.m. to 0.8 p.p.m., although 0.8 p.p.m. should be considered the upper limit and should not be maintained for long periods.

Within the allowable range of concentration, the copper induces the fish to secrete a copious

amount of mucus which causes the parasites to become detached. After they are sloughed from the body and settle to the bottom, cell division takes place and development proceeds normally to the free-swimming dinoflagellate stage, and at this point the copper sulfate apparently becomes lethal to them. Since it takes about seven days for the *Oodinium* organisms to develop into free-swimming dinoflagellates, it is necessary to maintain the copper concentration in the tank for at least that long. A ten-day treatment with copper sulfate is recommended.

Nigrelli (1936) reported that numerous marine fishes in the New York Aquarium were at one time heavily infected with *Oodinium ocellatum* and that the infection was not confined to the gills but was found on almost any part of the body. On fishes collected in the vicinity of the Hawaiian Islands I have found *Oodinium* only on the gill filaments. However, in May, 1954, some very sick Clown Fish (*Amphyprion percula*) that had been collected near Singapore were brought to the Aquarium for examination, and both gills and body were found to be covered with *Oodinium*. Treatment with copper sulfate solution was begun immediately. Two days later the parasites had disappeared from the body and gills. These fish were covered with tiny pit marks caused by the parasitic organisms, and one fish, more heavily pitted than the others, died two days later from severe fungus infection apparently resulting from the minute skin punctures. The others lived for several months after treatment, without recurrence of *Oodinium*.

Treatment of Freshwater *Oodinium* Infestations

Oodinium limneticum, a species described by Jacobs (1946), attacks many species of exotic freshwater fishes and causes a malady known to fish fanciers as velvet disease. Jacobs states that this is the first parasitic dinoflagellate known to attack freshwater fishes. It may occur on all external portions of the body, including the fins, trunk, eyes, mouth and gills. A fish parasitized with *O. limneticum* somewhat resembles one with an *Ichthyophthirius* infection, and because of this the disease may be mistakenly diagnosed; microscopic examination is necessary in order to make a positive diagnosis. Treatments most commonly used to cure *Ichthyophthirius* disease usually do not cure fishes with velvet disease.

While this paper was being prepared, velvet disease occurred only once in the Steinhart Aquarium. In this instance a tank of Glassfish (*Chanda lala*) became infected. Copper sulfate was added to the tank at a 0.5 p.p.m. level and two days after treatment was begun the disease disappeared completely. The fish suffered no ill effects from the copper. However, since our experience is so limited, we recommend caution when treating tropical freshwater fish with copper sulfate.

Determination of Concentration of Copper

When treating fish with copper sulfate, it is extremely important to make a daily chemical analysis of the water in the treatment tanks so that a constant level of copper may be maintained. In calculating the amount of copper sulfate needed to make up a solution containing a given amount of the metal, one must take into consideration the fact that the copper represents approximately only 25% of the total weight of $CuSO_4 \cdot 5H_2O$. To determine the amount of $CuSO_4 \cdot 5H_2O$ that must be added to a given volume of water in order to produce a desired concentration of copper in p.p.m., the following equation may be used:

$$x = \frac{v \times p \times 3.93}{1000}.$$

x = weight of $CuSO_4 \cdot 5H_2O$ in grams.
v = volume in liters.
p = parts per million of copper desired.
3.93 = number of grams of $CuSO_4 \cdot 5H_2O$ containing 1 gram of copper.

Copper does not long remain in solution in the presence of excess amounts of carbon dioxide and carbonates; therefore, if practicable, the fish to be treated should be transferred to a clean, nonmetallic tank devoid of all coral, shells, etc. If these substances are present, the copper may react with them to produce insoluble carbonates. Should it not be practicable to remove the fish from such an environment, copper sulfate may have to be added daily to compensate for the loss through precipitation.[1] If freshwater fishes infected with *Oodinium limneticum* are being treated, it is important to know whether the water is hard or soft. Copper is readily precipitated from hard water.

The concentration of copper in water may be determined by a colorimetric method using sodium diethyldithiocarbamate as the indicator. The reagents necessary for this are prepared in the following ways:

Sodium diethyldithiocarbamate solution: Dissolve 1 g of $N(C_2H_5)_2\ CS_2Na$ in 100 ml of copper-free distilled water and keep in a bottle of dark glass protected from sunlight. Add

[1] Precipitation of copper may be substantially decreased by the addition of citric acid to the copper sulfate solution before it is added to sea water. One part by weight of citric acid to 100 parts of copper sulfate usually suffices.

NH_4OH until the pH reaches 9.6-10. This retards decomposition of the carbamate. This reagent will remain stable for approximately 40 days when stored at this pH in a dark place.

Copper-free distilled water: Redistill distilled water, using an all-glass still.

Copper sulfate standard solution: Dissolve 0.393 g of $CuSO_4 \cdot 5H_2O$ in 1 liter of distilled water (copper-free). Dilute 25 ml to 250 ml. One ml of the diluted solution contains 0.01 mg cu.

Ammonium citrate buffer 20%: Dissolve 200 g of citric acid in about 500 ml of copper-free distilled water. Add C.P. NH_4OH until the pH reaches 9.0-9.2. Add copper-free distilled water to 1,000 ml.

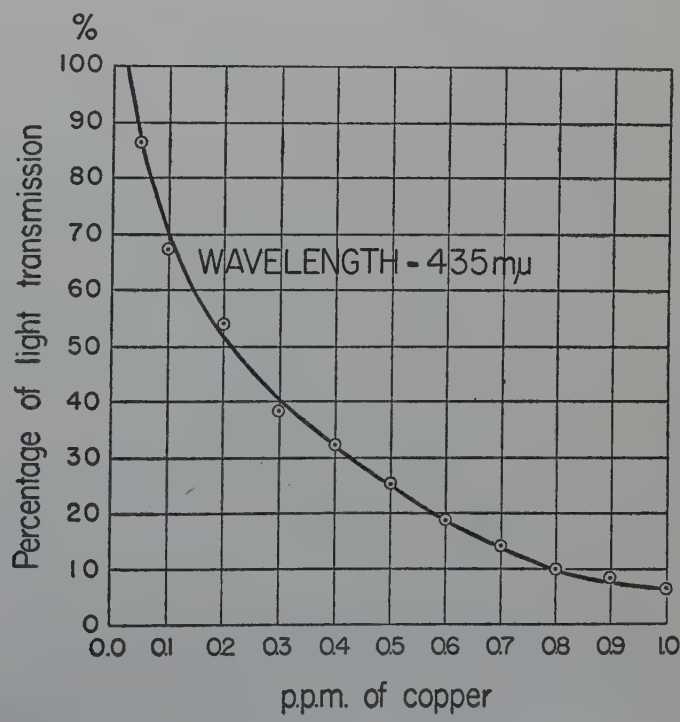
Carbon tetrachloride solution—reagent grade.

The detailed procedure for colorimetric analysis may be described as follows: In a separatory funnel, place 50 ml of the water to be analyzed, 5 ml of ammonium citrate buffer, and mix. Then add 1 ml of sodium diethyldithiocarbamate reagent and mix again. (The carbamate reagent, when introduced into the water sample containing copper, produces an amber color, the intensity of which is in direct proportion to the amount of copper present). To this mixture add exactly 10 ml of carbon tetrachloride and shake for at least 2 minutes to extract the color completely. The solution should be allowed to stand for 10 minutes to achieve complete phase separation. Carefully drain off the colored layer into a test tube and read in the colorimeter.[2] The colorimeter must have been previously adjusted so as to read 100 percent light transmission when a test tube containing the reagent grade carbon tetrachloride has been inserted. To interpret the readings in the colorimeter, it is necessary to refer to a calibration graph based on water samples containing known amounts of copper (Text-fig. 1). These water samples are prepared by adding carefully calculated quantities of the standard copper sulfate solution to 50 ml quantities of copper-free distilled water. The samples are then analyzed in the colorimeter and the readings plotted. Several colored carbon tetrachloride samples were measured at different wave lengths of light, and maximum light absorption was observed at 415 to 450 mu. Table 1 indicates that a wave length of 435 would be ideal. This agrees closely with the findings of Chow & Thompson (1952).

Discussion

It is probable that there is wide variation among different species of fishes in their tolerance to copper. Some tropical marine species begin to show distress at 1 p.p.m. and salmonoid fishes are adversely affected by concentrations

[2] A Bausch & Lomb spectronic twenty colorimeter was used for this purpose.



Text-fig. 1. Calibration graph indicating the percentage of light transmission through samples of known concentration of copper-diethyldithiocarbamate. Samples extracted with carbon tetrachloride from distilled water.

TABLE 1. PERCENTAGE OF LIGHT TRANSMISSION OF DIFFERENT WAVE LENGTHS OF LIGHT THROUGH VARIOUS CONCENTRATIONS OF COPPER-DIETHYLDITHIOCARBAMATE SOLUTION. SAMPLES EXTRACTED WITH CARBON TETRACHLORIDE AND MEASURED WITH B. & L. SPECTRONIC TWENTY COLORIMETER.

	Wave Length							
p.p.m. of copper	375 mu	400 mu	415 mu	425 mu	435 mu	450 mu	475 mu	500 mu
.05	97%	92%	88%	87%	86%	89%	94%	98%
.1	95	82	72	69	67	73	85	90
.2	91	75	60	55	54	61	75	88
.3	89	65	46	39	38	46	66	82
.4	87	60	38	32	32	39	61	76
.5	84	53	31	26	25	32	55	73
.6	82	48	25	20	19	24	47	69
.7	81	43	22	15	14	19	42	65
.8	74	36	16	11	10	15	37	58
.9	71	33	13	9	8	12	31	57
1.	70	29	11	7	6	11	30	54

of less than 0.5 p.p.m. Brook Trout fingerlings will die in concentrations above 0.1 p.p.m.

Oodinium disease appears to be very widely distributed throughout the tropic seas and has been reported from aquariums in different parts of the world. The Director of the Taraporevala Aquarium at Bombay reported in 1954 that this gill disease had affected some of the fishes on exhibition there, and the Honolulu Aquarium is periodically invaded by *Oodinium*. Reports of *Oodinium* disease have also come from the Zoological Society of London's Aquarium, the oceanarium (Marine Studios) at Marineland, Florida, and the New York Aquarium. Although this dinoflagellate seems to occur primarily in warm water, it invaded the temperate water system in the Steinhart Aquarium in at least one instance. Shortly after the severe attack on reef fishes in 1951, it was found on some of our local coastal fishes. Several Striped Bass (*Roccus saxatilis*), Rubberlip Seaperch (*Rhacochilus toxotes*) and Lingcod (*Ophiodon elongatus*) died from the disease. The water temperature in their tanks was approximately 65° Fahrenheit at the time of infection. It should also be noted that Nigrelli (1936) found *Oodinium* on fishes collected in Sandy Hook Bay, New Jersey, during the summertime. From this evidence it does not seem unreasonable to believe that this gill disease could invade the cooler waters along the California coast and cause serious damage to an important food fishery.

SUMMARY

At the Steinhart Aquarium, copper sulfate, in concentrations ranging from 0.4 p.p.m. to 0.8 p.p.m., has been found relatively non-toxic for tropical marine fishes, yet effective in eradicating the gill and skin infections of the dinoflagellate, *Oodinium*. Because of the toxicity of higher concentrations of copper, it is essential to control the amount present in the aquarium water. To make this possible a colorimetric method of determining the quantity of copper present in water is described. Velvet disease, which is caused by a freshwater dinoflagellate, *O. limneticum*, also appears to be cured by treatment with copper sulfate.

ACKNOWLEDGEMENTS

I wish to extend my thanks to Dr. Earl S. Herald, Curator of Aquatic Biology, Steinhart Aquarium, under whose direction the present work was done, for his interest, advice and sound judgment. I should also like to express my appreciation to Dr. Albert E. Bagot, Chemist, North Point Sewage Treatment Plant, San Francisco, for his assistance in working out a simplified method for copper determination in sea water, and to Dr. Jerald A. Ballou, Associate Professor of Physical Sciences, San Francisco State College, for his helpful advice regarding all chemical problems.

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EXPLANATION OF THE PLATE

Plate I

Fig. 1. Gill arch of a fish, showing *Oodinium* parasites clinging to the gill filaments.

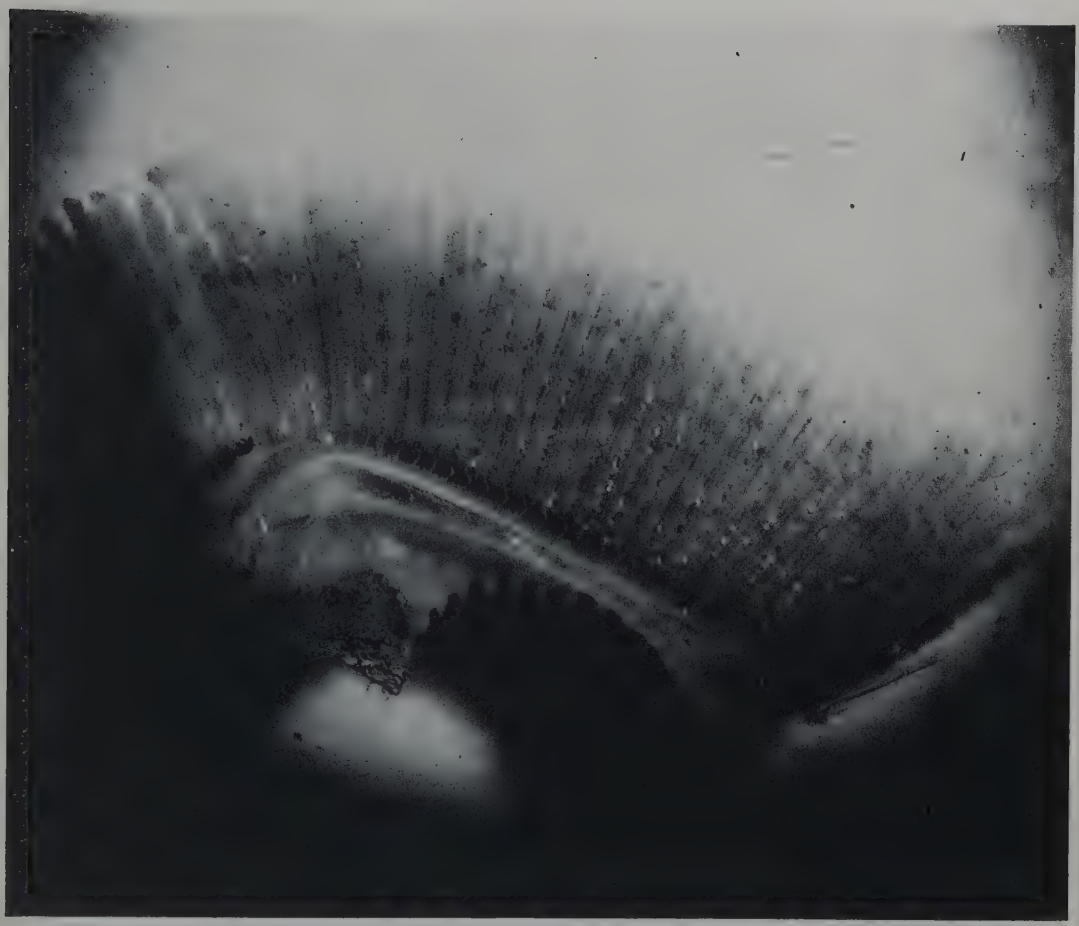

FIG. 1

THE USE OF COPPER SULFATE AS A CURE FOR FISH DISEASES CAUSED BY PARASITIC DINOFLAGELLATES OF THE GENUS OODINIUM

13

Polymorphism in Reared Broods of *Heliconius* Butterflies from Surinam and Trinidad[1]

WILLIAM BEEBE

Department of Tropical Research,
New York Zoological Society, New York 60, N. Y.

Plates I-VI

[This paper is one of a series emanating from the tropical Field Station of the New York Zoological Society at Simla, Arima Valley, Trinidad, British West Indies. This station was founded in 1950 by the Zoological Society's Department of Tropical Research, under the direction of Dr. William Beebe. It comprises 200 acres in the middle of the Northern Range, which includes large stretches of undisturbed government forest reserves. The laboratory of the station is intended for research in tropical ecology and in animal behavior. The altitude of the research area is 500 to 1,800 feet, with an annual rainfall of more than 100 inches.

[The present paper is chiefly concerned with the results of an eighteen-day trip to Surinam, which was undertaken in mid-April, 1954. Headquarters, as on a previous visit in 1953, were at the Moengo mine of the Surinaamsche Bauxite Maatschappij, where we were the guests of Mr. and Mrs. H. Meijer. The trip was made possible by the generous gifts of Mr. C. R. Vose, and by the cooperation of the Alcoa Steamship Company.

[The major part of the actual collecting of living *Heliconius* material in 1954 was undertaken by Henry Fleming, and the painstaking care of the eggs, larvae, pupae and ultimate emergence of the imagos was borne by Jocelyn Crane and Rosemary Kenedy. Future papers will deal with immature stages and rearing methods. For details of keeping and maintaining adult heliconids, see "Construction and Operation of Butterfly Insectaries in the Tropics," Crane & Fleming (Zoologica, 1953, Vol. 38, No. 14, pp. 161-172.)]

Forty years ago two able entomologists, J. J. Joicey and W. J. Kaye, made a thorough study of a collection of butterflies from French Guiana. The three paragraphs below are from their paper (1916):

"The following account is concerning a collection made during the months July, August and September, 1915, between the places St. Jean and St. Laurent on the Maroni River in French Guiana. The distance between the two places is about twelve miles or rather less, and the distance of St. Laurent (the nearer place) from the coast is about twenty miles. The collection, which contained numbers of specimens of other families, was, however, chiefly remarkable for the vast numbers and variety of forms of *Heliconius melpomene* and *Heliconius erato*. A few other species of *Heliconius* were obtained, but only a very few specimens of each.

"As it is, there are 731 specimens, which show a most wonderful range of variation. Many forms are new, and others graduate completely into these as well as to all the other known forms that have ever come from French Guiana.

"In comparison with the very large numbers of *melpomene* specimens the number of *erato* forms is small, being only 155 against 731 *melpomene*."

The object of these quotations is to emphasize several facts. First, the closeness of the locality to that in which we collected. The Moengo Mine Road is 30 miles in length, running east and west, beginning at Moengo and ending on the bank of the Marowyne (=Maroni) River at Albina, directly opposite St. Laurent on the French side. Our collecting was all done along the central 10 miles of the Moengo-Albina Road. The two collecting areas are thus only a few miles apart, separated by merely a change in direction and the width of the Marowyne River.

Secondly, the identification of the insects by Joicey & Kaye, insects with such a bewildering maze of colors and patterns, was necessarily based on individuals of unknown parentage or inter-relationship. The result of this was a plethora of bi- and trinomial names divided into

[1]Contribution No. 953, Department of Tropical Research, New York Zoological Society.

Offspring Numbers One to Five were reared on Surinam passiflora, and showed an average wing spread of 77.4 mm. The larvae of Numbers Six to Ten were fed on passiflora from Simla, Trinidad, and are uniformly smaller, showing an average wing spread of 63.4 mm. This dissimilarity in size bears no relation to variation in relative shape or size of the scarlet band. Were the 10 individuals not members of the same brood, 4 of their band variations would be considered worthy of some degree of recognition.

Major characteristics of the six broods may be summarized as follows, omitting lesser details of color and pattern:

Heliconius erato group

Brood A: Female parent: red radiations on fore- and hindwings; broken forewing red band. Male unknown.

Offspring: 1 is rayed like parent; forewing band represented by small, scattered, whitish spots.

Brood B: Male parent (Surinam): red radiations on fore- and hindwings; forewing band represented by a broken area of large, scattered, creamy spots.

Female parent: (Trinidad): red forewing band only.

Offspring: 13 with red radiations on fore- and hindwings; broken, red, forewing band.

Brood C: Female parent: red forewing band only. Male unknown.

Offspring: 2 like parent.

1 with broken, red, forewing band; red radiations on fore- and hindwing.

1 with broken, red, forewing band with whitish spots; red radiations on fore- and hindwing.

Brood D: Female parent: red forewing band only. Male unknown.

Offspring: 8 like female parent; red forewing band varying from solid to broken.

5 with red variations on fore- and hindwings; red forewing band varying from solid to broken.

Brood E: Female parent: red forewing band only. Male unknown.

Offspring: 4 like female parent.

Heliconius melpomene group

Brood F: Female parent: red forewing band only. Male unknown.

Offspring: 10 like female parent, with much variation in red band.

Summary

Six broods of heliconid butterflies were reared from parents taken at Moengo, Surinam. The one exception was a brood of 13, with the following parents: a male Surinam *Heliconius erato amazona,* mated to a female Trinidad *Heliconius erato hydara.*

Four broods of 1, 4, 4 and 13, had typically black-hindwinged, *Heliconius erato,* Surinam, female parents. The sixth brood of 10 had a *Heliconius melpomene,* Surinam, female parent.

The distinction between *Heliconius erato* and *melpomene* was established by means of differences in the larvae, as well as by the scent scales of the male offspring.

The offspring frequently differed radically both from the parent and from one another. These differences were tentatively correlated with illustrations in A. Seitz, "Macrolepidoptera of the World," Vol. 5, plates: "The American Rhopalocera," plate 78. New sibling relationships were therefore established for types of individual patterns and colors, to which have heretofore been applied terms such as species, subspecies, variations, forms, types, stages and aberrations.

Further data and interpretations are anticipated in cross-breeding future broods, resulting in F_2 generations.

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EXPLANATION OF THE PLATES

The light wing markings on the figures of all the plates are red or scarlet, with the following exceptions:

Plate I. Lower Figure: Pale spots on forewing are Light Lemon Yellow (Ridgway).

Plate II. Upper Left Figure: Forewing spots are Pale Green-Yellow (Ridgway).

Plate III. Lower Right Figure: The three pairs of light spots on forewing are white.

PLATE I

Brood A. *Heliconius erato* group

UPPER FIGURE. Female parent.
LOWER FIGURE. Single offspring.

PLATE II

Brood B. *Heliconius erato* group

UPPER LEFT FIGURE. Male parent.
UPPER RIGHT FIGURE. Female parent.
LOWER FIGURES. Thirteen offspring.

PLATE III.

Brood C. *Heliconius erato* group

UPPER FIGURE. Female parent.
LOWER FIGURES. Four offspring.

PLATE IV

Brood D. *Heliconius erato* group

UPPER FIGURE. Female parent.
LOWER FIGURES. Thirteen offspring.

PLATE V

Brood E. *Heliconius erato* group

UPPER FIGURE. Female parent.
LOWER FIGURES. Four offspring.

PLATE VI

Brood F. *Heliconius melpomene* group

UPPER FIGURE. Female parent.
LOWER FIGURES. Ten offspring.

POLYMORPHISM IN REARED BROODS OF HELICONIUS BUTTERFLIES
FROM SURINAM AND TRINIDAD

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FROM SURINAM AND TRINIDAD

14

Formation of a Mucous Envelope at Night by Parrot Fishes

HOWARD ELLIOTT WINN

The American Museum of Natural History, New York[1]

(Plate I)

INTRODUCTION

IT is well known that many parrot fishes rest on the bottom at night while leaning against various objects such as rocks, coral or shells. During the summer of 1954 at the Lerner Marine Laboratory, Bimini, B. W. I., several species of these fishes were observed in such positions at night with a large and conspicuous mucous fold around their bodies (Plate I). The formation of this envelope apparently represents a specialized function of the mucus-secreting system. When the parrot fishes, surrounded by mucus, were first observed in an aquarium at night, it was thought that this condition was pathological, but their respiration and reactions appeared normal. After repeated observations of this envelope formation, it became clear that this was normal behavior. Observations were made with a flashlight, and the transparent folds were difficult to see until the light rays were directed at an angle which made them more easily visible.

C. M. Breder, Jr., Priscilla Rasquin, Louise Stoll and Carolyn Winn kindly read and made suggestions on the manuscript, which are gratefully acknowledged.

OBSERVATIONS

Five species of parrot fishes were placed in laboratory aquaria. Four of these, *Scarus croicensis* Bloch, *S. punctulatus* Cuvier & Valenciennes, *Pseudoscarus guacamaia* (Cuvier), and *Sparisoma pachycephalum* Longley,[2] formed conspicuous mucous folds only in the dark, except under certain special conditions. The nomenclature is that used by Longley & Hildebrand (1941). Five individuals of *S. croicensis* were also observed at night in dead finger coral (*Porites* sp.) branches beside the laboratory dock by means of goggles and an underwater flashlight. All were surrounded by a mucous sheath. One species, *Sparisoma chrysopterum* (Bloch & Schneider), did not form such a structure in aquaria or in the water off the dock, where two individuals were observed. The standard length of the various fish studied varied from about 6 to 21 cms. The water temperature in the aquaria was 30 to 32° C.

The structure of the mucous envelope was similar in the four species that produced it. It started as a fold at the mouth and went backward to surround the body of the fish completely. A little flap with a hole in its center covered the open mouth and moved in and out as the fish breathed. Posterior to the caudal fin, an opening of one to several centimeters in diameter was present through which the expired water left. Thus, a flow of water over the gills was insured. As Plate I shows, the folds were complex and extended up to several centimeters away from the body, depending on the size of the individual. The envelope seemingly consisted of a series of layers of mucus and varied in shape to some extent. In a specimen of *S. punctulatus,* about 18 cms. in standard length, the maximum length, width and depth of the envelope was 25 × 13 × 9 cms. In some instances the mucus near the bottom had sand grains attached to it and silt particles often settled on the upper part, which made the envelope more readily observable.

The fish usually leaned against the aquarium wall or drainpipe. However, they swam into conch shells or in among the branches of finger coral which they used for support if available. The mucus was then secreted in these positions.

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[2]The identification of the individual used, which was 6 cms. in standard length, is only tentative.

In one instance, two *S. croicensis* leaned against each other, one being against the aquarium wall, and formed what appeared to be a single fold around both of them.

The mucus was transparent and gelatinous. Large amounts could easily be picked up in the hand. Sometimes when a fish moved out of the fold, the structure partially collapsed into a large ball (Plate I, Fig. 2), but at other times it remained temporarily in the expanded condition.

The exact formation of the envelope was not observed because a light could only be turned on for a few seconds every five minutes or so. If the light was left on, the fish stopped the secretion and soon became active enough to break out of the fold. It appeared to form first around the head region and then pass back over the body, but it was not determined what groups of mucus cells were involved.

The consistency of the formation of the structure was established by nightly observation for the different fish as follows: four individuals of *S. croicensis* and one of *S. punctulatus* from July 19 to 28; five *S. croicensis* from August 17 to 27; one *S. punctulatus* from August 18 to 23; and one *P. guacamaia* from August 21 to 27. Other individuals were watched only occasionally.

Certain differences were noted in the time that some species required to form the completed envelope in the dark and the time required to break out of the folds after the lights were turned on. Although all the species were not studied comparatively, it was demonstrated that *S. croicensis* took longer to form the folds and broke out more quickly than either *S. punctulatus* or *P. guacamaia.* The individuals of *S. croicensis* normally took more than one hour to complete the fold. Under 0.3 footcandles of light, one individual formed it in 80 minutes and another between 75 and 145 minutes. Under these conditions the other two species completed the envelope usually within 30 minutes (*P. guacamaia* in 20 mins., *S. punctulatus* in 25 and 30 mins.). When the lights were turned on, *S. croicensis* usually broke out in less than 30 minutes (5, 10, 25 and 28 mins.), whereas the other two species usually required a longer period (*P. guacamaia* more than 30 mins.). It should be noted, however, that two specimens of *S. punctulatus* and *P. guacamaia* were considerably larger than those of *S. croicensis.*

At night the respiratory rate of the parrot fishes was considerably reduced. One *S. punctulatus* inspired 76 and 73 times per minute during the day and 47 times at night. One *S. croicensis* inspired 100 and 144 times per minute during the day and 56 times at night.

In several instances, under apparently anoxic conditions, some of the parrot fish formed the mucous envelope around their bodies in daylight. One specimen of *S. pachycephalum* and three of *S. croicensis* were observed to do this in aquaria where the running water supply had shut off. The water had been turned off about an hour and the temperature of the water had increased in two tanks each containing two fish. One specimen of *P. guacamaia* (about 21 cms. st. l.), presumably in a state of anoxia in a bucket, formed the envelope. In at least one instance, three individuals of *S. croicensis,* not under anoxic conditions but wedged in finger coral, formed the structure in daylight. It was also usually produced in tanks darkened during the day, although the formation time was about doubled.

When a light is turned on after the slime covering is formed, the fish breaks out in such a manner as to indicate that the envelope is sufficiently resistant to modify the first swimming movements. In 4 out of 7 instances, parrot fishes moved backwards out of the mucous fold. Twice they wiggled out sideways and once one swam forward out of it.

In one instance, the mucus at the front of a parrot fish was separated from the mouth. The fish then moved forward slightly so that the mucus again came in contact with the mouth and a new hole was made for breathing.

Discussion and Summary

The formation by parrot fishes of a large mucous envelope after dark is a remarkable behavioral trait that evidently has not been previously recorded. It has been observed both in aquaria and in the field. Four species apparently do this normally every night, whereas *Scarus brachiale* does not form the envelope. Most fishes are covered with a thin coat of mucus and its secretion is a slow, possibly continuous process. The behavior recorded here is a divergence from this pattern and seems to represent a rare specialization of the mucus-secreting system. More refined chemical differences may be present, but these have not been adequately investigated.

The structure has been described in the previous section. The fish utilize any object to swim into or lean against at night before the mucus is secreted. Precisely how the envelope is formed has not been determined. At the resumption of daytime activities the fish's behavior is modified in that the fish has to back out or wiggle vigorously forward to rid itself of the mucous coat. Preliminary data show that *S. croicensis* takes longer to form the structure and takes less time to break out than either *S. punctulatus*

or *Pseudoscarus guacamaia*. This seems to indicate that the latter two species have a higher threshold of reactivity to light stimuli under these conditions. The ecological significance of these differences is unknown. Two questions that arise are the nature of the physiological mechanisms involved and the function of this conspicuous mucous envelope.

Reid (1894) and Uhlich (1937) indicated that there is a direct reaction to touch or chemical stimulation of the mucus cells and possibly a nervous control. However, as they indicate, their experiments of direct stimulation of intact and excized skin do not seem to present critical evidence bearing on the latter conclusion. The reactions of parrot fishes to light and anoxia strongly suggest that there is a nervous control, at least in these fishes, although the possibility of hormonal influence cannot be eliminated. The lack of light, which is usually necessary for the formation of the envelope, leads one to believe that the stimulus is mediated through the eyes to the nervous system and finally to the mucus cells. However, it appears that anoxic conditions produce the same effect and presumably stimulate the respiratory system which relays to the nervous system. When in the dark, the fish's respiration is considerably lowered so that there may not be transmission through the optic system to the mucus cells, but instead the stimulus may be mediated by the respiratory system because of the reduced oxygen supply as under anoxic conditions.

The function of the mucous envelope is not clear and can only be speculated upon at this time. The envelope may protect the fish from nocturnal predators, since it would be particularly vulnerable when lying on the bottom. Protection against the settling of silt is one of several other possibilities. The nature of the mucous secretion in the parrot fishes may make it possible to test some of these supposed functions in future experiments.

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EXPLANATION OF THE PLATE

FIG. 1. Large mucous envelope around a rainbow parrot fish (*Pseudoscarus guacamaia*), standard length, 21 cms.

FIG. 2. On the left, mucous fold around a parrot fish, *Scarus croicensis*. On the right, another individual has freed itself from the mucous fold, which remains as a nearby oval mass of slime.

FIG. 1

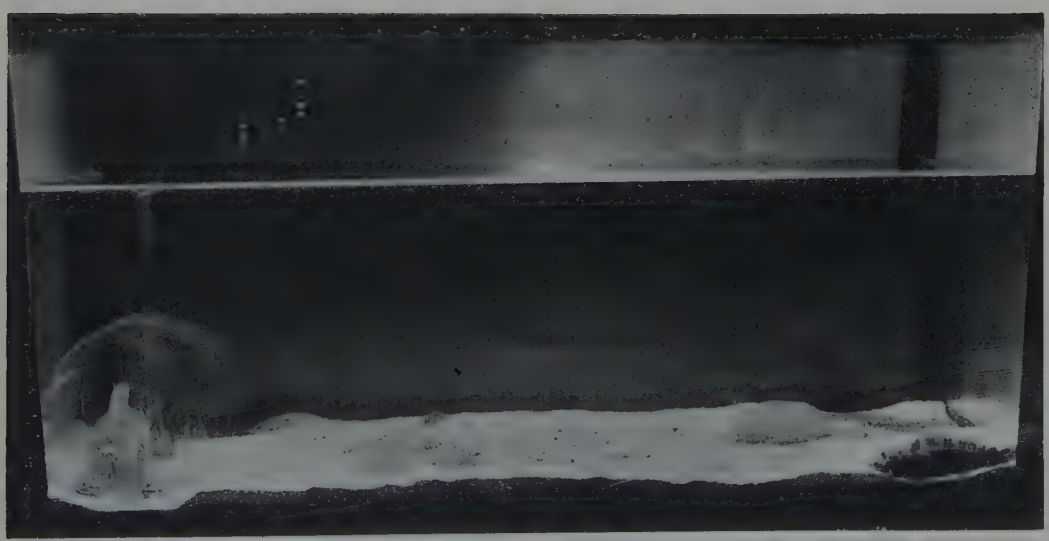

FIG. 2

FORMATION OF A MUCOUS ENVELOPE AT NIGHT BY PARROT FISHES

15

A Revision of the Surgeon Fish Genus *Ctenochaetus*, Family Acanthuridae, with Descriptions of Five New Species[1]

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University of Hawaii, Honolulu 14, T.H.

(Plates I & II; Text-figures 1-3)

THE genus *Ctenochaetus* is distinguished from other surgeon fish genera chiefly by its unique teeth, which are numerous, loosely attached in the jaws and very elongate, with expanded incurved tips. Little difficulty is experienced in assigning specimens to this genus; however, insufficient attention has been given to identification at the species level. Two specific names, *striatus* Quoy & Gaimard and *strigosus* Bennett, have been applied indiscriminately. Generally only one or the other name is used and some authors have asserted their belief that the genus is monotypic. In this paper the validity of *C. striatus* and *C. strigosus* is established and five other species are described as new.

Several species of *Ctenochaetus* are very abundant in the tropical inshore waters of the Pacific and Indian Oceans and the genus is extensively recorded in the literature. Many records are listings of names only and others contain too little descriptive information to provide identification. Such records have been omitted, generally, unless the specimens reported on were examined by me.

Drawings of adults of only two of the seven species are given. The others have either been pictured previously or present insufficient differences in external appearance from remaining species to warrant the preparation of figures. The shape of the teeth (principally the number of denticulations on the lateral edge of the expanded tips) has been found to be the most important basis for the separation of species. Text-fig. 1 consists of drawings of the teeth of all seven species. The number of teeth (Text-fig. 2) and fin ray and gill raker counts (Tables 1-3) are also of diagnostic value.

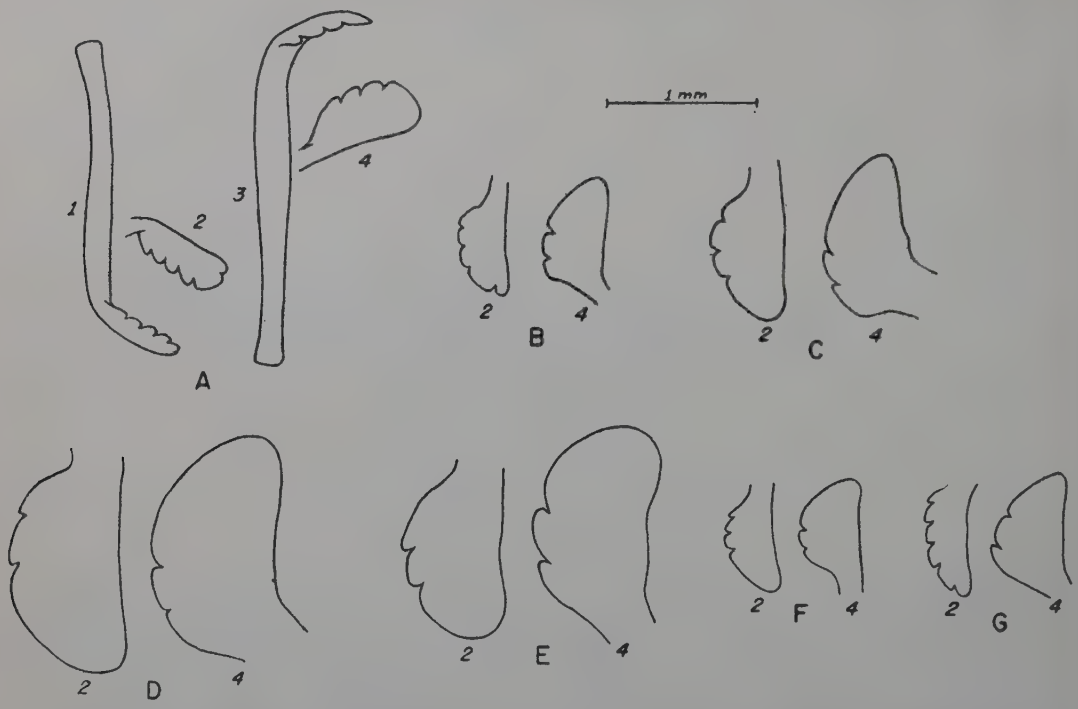
It is with pleasure that I acknowledge the guidance and assistance given by Leonard P. Schultz, Ernest A. Lachner and Robert H. Kanazawa of the United States National Museum, where much of the work on this paper was done. William A. Gosline of the University of Hawaii provided helpful counsel, and thanks are due Boyd W. Walker of the University of California at Los Angeles and George S. Myers of the Stanford Natural History Museum for the loan of specimens.

The types of the new species are deposited in the United States National Museum.

Methods of Counting and Measuring

Each ray of the dorsal and anal fins with a distinct base was counted regardless of how close adjacent rays might be. In cases where two rays branch from a common base, they were counted as one. At times dissection was necessary to determine whether the last two rays originate from a single basal element. Pectoral fin ray counts include the two uppermost unbranched rays, the first being a very short bony spicule. Few gill raker counts were made, because it is difficult to remove a complete gill arch from an acanthurid without damaging the specimen. The gill rakers are small and occur in two distinct series, anterior and posterior (actually more medial on the arch than posterior). All rakers (including rudiments) in each series were counted separately. In many instances it was not possible to determine which raker occupied the position at the angle of the arch, therefore

[1] Contribution No. 58, Hawaii Marine Laboratory, in co-operation with the Department of Zoology and Entomology, University of Hawaii.



TEXT-FIG. 1. Camera lucida drawings of the teeth of *Ctenochaetus*. A. *striatus*, 141 mm. specimen, Marshall Islands. B. *strigosus*, 119 mm. specimen, Hawaiian Islands. C. *cyanoguttatus*, type. D. *hawaiiensis*, type. E. *magnus*, type. F. *tominiensis*, type. G. *binotatus*, type. 1. lateral view of upper tooth. 2. inner view of end of upper tooth. 3. lateral view of lower tooth. 4. inner view of end of lower tooth. All of the teeth were taken from the left side of the jaw near the center of the mouth.

only the total count is recorded. Occasional specimens showed a fusion of rakers on the dorsal end of the medial series where the rakers normally become broad, low ridges; no counts from such specimens are included in the table. At least for *C. striatus*, no obvious increase in gill raker counts occurs with increasing size after the fish have completely transformed from the late postlarval or acronurus stage. The state of being completely transformed is easily distinguished by the presence of fully formed scales on the body.

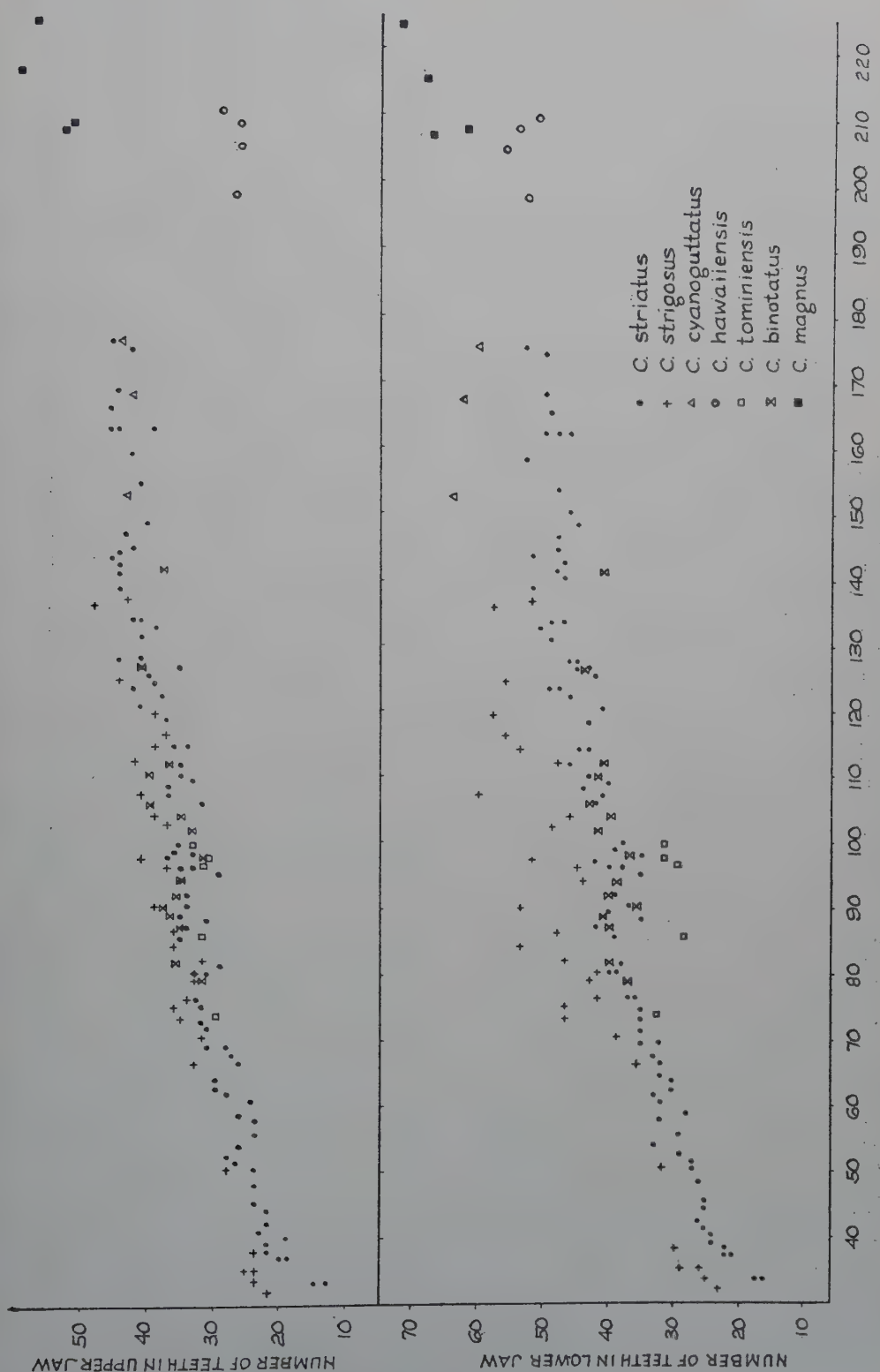
The standard length was measured from the tip of the snout to the posterior end of the hypural plate. Head length was taken from the tip of the snout to the most posterior part of the opercular membrane. Body depth is the distance from the natural groove at the base of the second anal spine to a similar groove at the base of the dorsal fin. Caudal concavity is the distance between vertical lines passing through the tips of the shortest middle caudal ray and the longest ray of the dorsal lobe of the caudal fin. Proportional measurements are based on specimens above 70 mm. in standard length.

Counts of the number of teeth were not made in specimens with tightly closed or damaged jaws. Even the best specimens generally had missing teeth; however, these were detected by empty sockets in the gums and by gaps in the series and were included in the total counts.

The number of teeth was found to increase with increasing size in *striatus* and *strigosus*. Text-fig. 2 represents a graph of the relationship between the number of teeth and the standard length of these two species. Such data from the available specimens of the other five species are also included in the graph, but there are not enough of these of different lengths to clearly show this relationship.

Scale counts are difficult to make, for the scales are small and not in even rows. Approximate scale counts are recorded as the number of vertical scale rows from the upper end of the gill opening to the posterior end of the caudal peduncle spine.

In the description of new species, data in parentheses are referable to paratypes. Proportional measurements and counts of scales were made on five paratypes, if available, including the largest and smallest specimens. Fin ray counts are based on all paratypes. Tooth counts



TEXT-FIG. 2. Tooth counts of *Ctenochaetus*.

and gill raker counts of paratypes may be found in Text-fig. 2 and Table 3, respectively.

Genus Ctenochaetus Gill

Ctenodon Klunzinger, Synopsis der Fische des Rothen Meers, 1870: 509 (proposed as a subgenus; preoccupied by *Ctenodon* Wagler 1830).

Ctenochaetus Gill, Proc. U. S. Nat. Mus., **7**: 279. 1885 (Type species, *Acanthurus strigosus* Bennett).

Quoy & Gaimard (1824) described and figured the first species of this genus as *Acanthurus striatus*. They remarked on its unusual dentition. E. T. Bennett (1828) named another, *Acanthurus strigosus*, from Hawaii, and he accurately described the teeth. Although Günther (1861) separated two of the species, *strigosus* and *ctenodon* Cuvier & Valenciennes, in a key on the basis of dentition, he left them in *Acanthurus*. Gill (1884) established the genus *Ctenochaetus*, designating *A. strigosus* Bennett

Table 1. Counts of the Soft Rays of the Dorsal and Anal Fins for Species of *Ctenochaetus*

Species and locality	Dorsal soft rays								Anal soft rays							
	24	25	26	27	28	29	30	31	21	22	23	24	25	26	27	28
striatus																
Wake I.					3	6	4						2	6	4	1
Marshall Is.				1	14	39	8					1	3	34	23	1
Gilbert Is.				1	9	13	4	1				1	5	21	1	
Line Is.					2	1	1						1	2	1	
Phoenix Is.					2	2	1						1	3	1	
Samoa Is.					4	9	1	1						7	7	1
Tuamotu Arch.						2	7	2						6	5	
Society Is.						3	4						1	1	5	
Austral Is.							1								1	
Solomon Is.					2	1								3		
New Hebrides					1	1								1	1	
Palau Is.					1	1								1	1	
Mariana Is.					5	8	11	4					1	5	16	6
Philippine Is.				12	24	14						3	23	22	2	
Formosa					2									2		
East Indies				2	2	3							2	4	1	
Egypt, Red Sea					3	2							4		1	
Mauritius				2	2	2	1						3	2	2	
Madagascar							1								1	
strigosus																
Hawaiian Is.		4	19	23	1						9	32	6			
Johnston I.			8	1							2	6	1			
Tuamotu Arch.			4						1	1	1	1				
Philippine Is. & East Indies		3	6	4							7	6				
Mauritius			1								1					
cyanoguttatus, sp. nov.																
Gilbert Is.				2									2			
Phoenix Is.					1								1			
Cocos I.					1								1			
hawaiiensis, sp. nov.																
Hawaii				1	3								3	1		
magnus, sp. nov.																
Malden & Jarvis I.				3								2	1			
Cocos I.			1									1				
tominiensis, sp. nov.																
Celebes	1	5								4	2					
binotatus, sp. nov.																
Philippine Is. & Molucca Is.	2	4	16	8						1	9	17	3			

TABLE 2. COUNTS OF PECTORAL FIN RAYS FOR THE SPECIES OF *Ctenochaetus*

Species and locality	Pectoral fin rays 15	16	17
striatus			
Marshall, Mariana, and Samoa Is.		6	29
Philippine Is. and East Indies		15	24
Egypt, Red Sea		3	4
Mauritius		3	1
strigosus			
Hawaiian Is.	1	22	
Philippine Is.		13	
Mauritius		1	
cyanoguttatus, sp. nov.			
Gilbert, Phoenix, and Cocos Is.		1	3
hawaiiensis, sp. nov.			
Hawaii		4	
magnus, sp. nov.			
Malden, Jarvis, and Cocos I.		1	3
tominiensis, sp. nov.			
Celebes	2	4	
binotatus, sp. nov.			
Philippine and Molucca Is.	3	27	

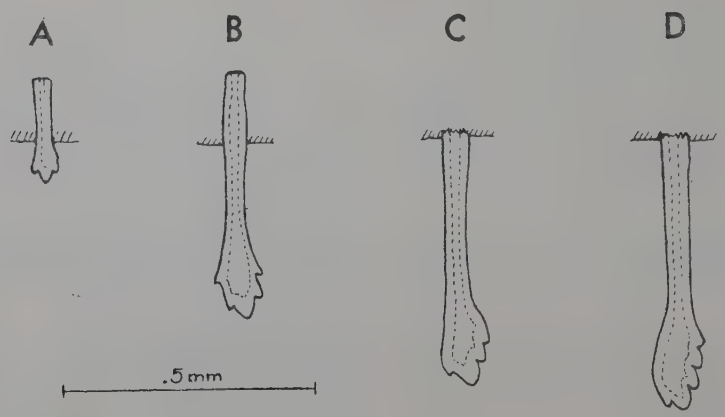
as the type species. He was apparently unaware of *A. striatus* Quoy & Gaimard. Prior to Gill, Klunzinger (1870) set up *Ctenodon* as a subgenus for *strigosus* and *ctenodon*. This was not the *Ctenodon* of Swainson (1839), the type species of which was *Acanthurus sohal* (Forskål), and possibly not of Bonaparte (1833) (for which there was no description nor type specified). However, Wagler's use in 1830 of *Ctenodon* for a reptile invalidates subsequent generic use of this name.

Ctenochaetus is characterized as follows: body compressed, elliptical, the depth contained 1.7 to 2.2 in standard length; head length 3 to 3.8 in standard length; caudal peduncle armed on each side by a single, sharp, folding spine; length of caudal peduncle spine 2.3 to 4.3 in head length; least depth of caudal peduncle 1.9 to 2.7 in head length; mouth small, terminal, and only slightly protractile; jaws equal; teeth numerous, in a single series in each jaw, movable, elongate, with tips expanded, incurved, and denticulate on the lateral margin; eye diameter contained about 3 to 5 times in head length; interorbital space 2.6 to 3.3 in length of head; gill openings restricted to the sides; gill membranes confluent and attached to the isthmus; scales ctenoid, very small, and not in regular rows; head scaled, though not conspicuously; lateral line complete; dorsal fin single, continuous, unnotched, with 8 spines, the first small and easily overlooked; dorsal soft rays 24 to 31; anal fin with 3 spines, the first very short, and 21 to 28 soft rays; the first 2 to 6 dorsal and anal soft rays unbranched; pectoral fins long, their length contained 2.7 to 3.2 in standard length; pectoral rays 15 to 17; pelvic fins I, 5, relatively long and pointed, and only slightly posterior in their insertion to the base of the pectoral fins; caudal fin with 16 principal rays and varying in shape from lunate to nearly truncate; 22 vertebrae; stomach subspherical to oval with very thick walls.

The single erectile spine on each side of the caudal peduncle is a prominent feature of four of the acanthurid genera, *Acanthurus, Ctenochaetus, Paracanthurus* and *Zebrasoma*. The latter genus differs from the other three in having 4 or 5 dorsal spines instead of 7 to 9. The monotypic *Paracanthurus* is readily separable with its pelvic count of I, 3 and its remarkable coloration. *Ctenochaetus*, as noted, is distinguished primarily by its unusual dentition. Usually the genus has 8 dorsal spines (in counts of over 300 specimens two had 9 and three had 7 dorsal spines). In the 28 species of Indo-Pacific *Acanthurus* the usual count of dorsal spines is 9. Two species, *A. pyroferus* Kittlitz and *A. sohal* (Forskål), have 8 and another, *A. nubilus* (Fowler & Bean), has 6 or 7.

Ctenochaetus striatus and certain other species of this genus are similar in general body shape and color pattern to *Acanthurus nigroris* Cuvier & Valenciennes (=*Hepatus atramentatus* Jordan & Evermann, 1905, and *Acanthurus lineolatus* Günther, 1873). The similarity may be seen even in the transforming acronurus stages of these fishes by comparing Fig. A (*C. striatus*) and Fig. C (*A. nigroris*) in Plate 62 of Schultz & Woods (1953). Other *Acanthurus* species such as *lineatus* and *sohal* may be striped in the postacronurus form, but the stripes do not angle downwards as they pass posteriorly on the body. Inconsistent with the apparent close relationship of *Ctenochaetus* to *A. nigroris* is the finding that the roundish, heavy-walled stomach of the former does not resemble the elongate, thin-walled stomach of *A. nigroris* but rather that of such acanthurids as *A. olivaceus* and *A. gahhm*.

Although no existing *Acanthurus* species may be considered the direct progenitor of the genus *Ctenochaetus*, it seems reasonable to conclude (as has Aoyagi, 1943) that *Ctenochaetus* was derived from *Acanthurus*-like stock. This view is supported by the fact that postlarval *Ctenochaetus* have teeth much like those of postlarval *Acanthurus*, and these transform into the typical adult *Ctenochaetus* dentition (see Text-fig. 3 and discussion under *striatus*).



TEXT-FIG. 3. A. Upper tooth of 27 mm. acronurus larva of *Ctenochaetus strigosus.* B to D. Upper teeth from 31 mm. postacronurus stage of *Ctenochaetus striatus.* Dotted lines indicate margins of pulp cavity.

The food habits and method of feeding of *Ctenochaetus strigosus* were investigated. The stomach contents of seven adult specimens from different localities in the Hawaiian Islands were analyzed. All but one fish contained a very large amount (up to 90%) of fine inorganic sediment; the remaining detrital material was mostly algal, consisting of diatoms and small fragments of many kinds of red, green and blue-green algae. About 1 to 2 per cent was soft, unidentifiable organic matter. There were occasional tiny molluscs and crustaceans, sponge spicules, holothurian plates, pedicellaria fragments, etc. The stomach of one specimen was filled primarily with a fine red alga (*Ceramium* sp.), though there was still a large amount of sediment. When a thallus of fine filamentous red algae (*Polysiphonia* sp.) was placed in an aquarium containing two adult specimens of *strigosus,* the fish attempted to feed upon it. Their slender movable teeth, not able to effectively bite off pieces, soon became tangled in the algae, resulting in very little being ingested. When fine particles of the alga were put in the tank and allowed to settle, the fish fed in the following manner: the body was elevated to a near-vertical position about 15 mm. above the bottom, there was a pause, then the fish pecked at a small area, the teeth and lips scraping over the surface. Such an area was not only cleaned of particulate algae but also of very fine sediment that had collected there, suggesting that a suction mechanism is involved as well as a scraping one. There was definitely no lateral plowing or sieving action by the teeth as their comb-like structure might suggest. Sand on the bottom was generally avoided, but if picked up, most was usually forcefully ejected.

KEY TO THE SPECIES OF CTENOCHAETUS

1a. No prominent blackish spot at base of last 3 to 7 rays of both the dorsal and anal fins. (Juvenile and young adult *striatus* have a small black spot basally at rear of dorsal fin only).

2a. Teeth of the upper jaw with 4 to 7 denticulations (including tip) on lateral edge of their distal expanded ends.

3a. Teeth of the upper jaw with 5 to 7 denticulations on lateral edge of their distal expanded ends; body with numerous pale longitudinal stripes (often faint or not visible in preserved specimens); spots, if present, occur only on head or anterior part of body; inter-radial membranes of pectoral fin hyaline; length of longest dorsal ray contained 3.6 to 4.4 times in standard length.

4a. Teeth of upper jaw with 6 denticulations (rarely 5 or 7); teeth of lower jaw with 4 denticulations (including tip); caudal fin lunate, caudal concavity (see section on methods of measuring and counting) contained 3.7 to 6 times in standard length; body depth contained 1.9 to 2.3 in standard length; dorsal fin rays VIII, 27 to 31 (usually 28 to 30); anal fin rays III, 24 to 28 (usually 25 to 27) . . . . . *Ctenochaetus striatus* (Quoy & Gaimard).

4b. Teeth of upper jaw with 5 denticulations; teeth of lower jaw with

3 denticulations (including tip); caudal fin moderately concave, caudal concavity contained 5.7 to 10 times in standard length; body depth contained 1.7 to 2.0 in standard length; dorsal fin rays VIII, 25 to 28 (usually 26 or 27); anal fin rays III, 21 to 25 (usually 23 or 24)..... *Ctenochaetus strigosus* (Bennett).

3b. Teeth of upper jaw with 4 denticulations on lateral edge of their distal expanded ends; body without stripes, when alive speckled with numerous bright blue spots which may or may not persist as pale spots in preserved specimens; inter-radial membranes of pectoral fin dark brown; length of longest dorsal ray contained about 5.2 times in standard length..... *Ctenochaetus cyanoguttatus,* sp. nov.

2b. Teeth of upper jaw with 3 denticulations (including tip) on lateral edge of their distal expanded ends.

5a. Ratio of number of teeth in lower jaw to number in upper jaw about 2:1; caudal fin slightly emarginate, caudal concavity contained 18 to 40 times in standard length; longest dorsal ray contained 4 to 5 times in standard length; inner surfaces of lips plicate, margins crenulate; distance from base of upper lip to distal end of upper teeth contained 3.1 to 3.7 times in head length; length of snout contained 3.6 to 3.9 in standard length..... *Ctenochaetus hawaiiensis,* sp. nov. (Plate II, Fig. 2)

5b. Ratio of number of teeth in lower jaw to number in upper jaw about 1.2:1; caudal fin lunate, caudal concavity contained 6 to 7 times in standard length; longest dorsal ray contained 5.5 to 6 times in standard length; inner surfaces and margins of lips smooth; distance from base of upper lip to distal end of upper teeth contained 4.5 to 5.3 times in head length; length of snout contained 4.1 to 4.8 in standard length.... *Ctenochaetus magnus,* sp. nov.

1b. A prominent blackish spot at base of last 3 to 7 rays of both the dorsal and anal fins, these spots extending narrowly on adjacent regions of caudal peduncle.

6a. Membranes of caudal fin and posterior parts of dorsal and anal fins pale; margins of lips papillate; enlarged distal curved portions of each tooth of upper jaw with lower half smooth and blade-like and upper half with 3 (rarely 2) lateral denticulations ... *Ctenochaetus tominiensis,* sp. nov.

6b. Membranes of caudal, dorsal, and anal fins dark brown; margins of lips smooth; enlarged distal curved portion of each tooth of upper jaw divided into 6 approximately equal lateral denticulations .... *Ctenochaetus binotatus,* sp. nov.

CTENOCHAETUS STRIATUS (Quoy & Gaimard)

Text-fig. 1 A; Plate I, D-F; Text-fig. 3 B-D

Acanthurus argenteus Quoy & Gaimard, Voy. *Uranie,* Zool.: 372, pl. 63, fig. 2. 1824 (Guam); Cuvier & Valenciennes, Hist. Nat. Poiss., **10**: 239. 1835.

Acanthurus striatus Quoy & Gaimard, Voy. *Uranie,* Zool.: 373, pl. 63, fig. 3. 1824 (type locality, Guam); Cuvier & Valenciennes, Hist. Nat. Poiss., **10**: 229. 1835 (not Hawaiian Is.); ?Günther, Cat. Fish. Brit. Mus., **3**: 334. 1861 (Borneo).

Acanthurus ctenodon Cuvier, Règne Animal., **2**: 224. 1829; Cuvier & Valenciennes, Hist. Nat. Poiss., **10**: 241, pl. 289, 1835 (Caroline Is. and New Guinea); Günther, Cat. Fish. Brit. Mus., **3**: 342. 1861 (Ceylon and East Indies); Bleeker, Ned. Tijdschr. Dierk., **1**: 156. 1863 (Halmahera); Playfair, Fishes of Zanzibar: 57. 1866 (Zanzibar) (as variety *a*); Day, Proc. Zool. Soc. London: 688. 1870 (Andaman Is.).

?Acanthurus flavoguttatus Kittlitz (not of Herre, 1936, or Fowler, 1944), Mus. Senckenb., **1**: 193, pl. 13, fig. 5. 1834 (Caroline Is.).

?Acanthurus Ketlitzii Cuvier & Valenciennes, Hist. Nat. Poiss., **10**: 222. 1835.

Ctenodon Cuvierii Swainson, Nat. Hist. Class. Monocard. Animals **2**: 256. 1839.

Acanthurus strigosus Cuvier & Valenciennes (not of Bennett), Hist. Nat. Poiss., **10**: 243. 1835 (New Guinea); Bleeker, Nat. Tijdschr. Ned. Indië, **4**: 264. 1853 (New Guinea, not Hawaiian Is.); *ibid.*, **6**: 102. 1854 (East Indies); ?Günther, Cat. Fish. Brit. Mus., **3**: 342. 1861 (New Guinea, not Hawaiian Is.); Kner, *Novara* Exped. Fische, **1**: 211. 1865-1867 (Tahiti); Günther, Journ. Mus. Godeffroy, **2** (3): 116, pl. 79. figs. B and C. 1873 (Indo-Pacific); Day, Fishes of India, **1**: 207, pl. 47, fig. 2. 1876 (Andaman Is.);—Fauna Brit. India, **2**: 143. 1889 (India); Hardenburg, De Tropische Natuur, **22**: 155, fig. 1. 1933.

Acronurus argenteus Günther (in part), Cat. Fish. Brit. Mus., **3**: 346. 1861 (Marianas).

Acanthurus (*Ctenodon*) *ctenodon* Klunzinger, Synops. Fische Rothen Meeres: 509. 1871 (Red Sea).

Acanthurus (*Ctenodon*) *strigosus* Klunzinger, Fische Rothen Meeres, **1**: 85. 1884 (Red Sea); Weber, *Siboga* Exped. Fische, **57**: 319. 1913 (Indo-Australian Arch.).

Ctenochaetus strigosus Seale, Occ. Pap. B. P. Bishop Mus., **1**: 109. 1901 (Guam); Fowler & Ball (in part), B. P. Bishop Mus. Bull., 26: 19. 1925 (Wake I., not Hawaiian Is. or Johnston I.); Herre, Philipp. Journ. Sci., **24**: 438, pl. 15, figs. 2 and 3. 1927 (Philippine Is.); Fowler (in part), Mem. B. P. Bishop Mus., **10**: 247. 1928 (Oceania, not Hawaiian Is.); Fowler & Bean (in part), Bull. U. S. Nat. Mus.,100, **8**: 200. 1929 (Philippine Is. and East Indies); Herre, Field Mus. Nat. Hist. Zool. Ser., **21**: 247. 1936 (Polynesia and East Indies); Fowler, Acad. Nat. Sci. Phila. Mon., 2: 76, 147, 173, 185, 202, 215. 1938 (Marquesas Is., Society Is. and Christmas I. in the Line Is.); Poll, Mus. Roy. Hist. Nat. Belgique, **18**: 11. 1942 (Society Is.); Aoyagi, Coral Fishes: 218, pl. 6, fig. 20 (teeth only). 1943 (Riu Kiu Is.); Schultz (in part), U. S. Nat. Mus. Bull., 180: 161. 1943 (Phoenix and Samoa Is.); Hiyama, Poisonous Fishes South Seas: 83, pl. 19, fig. 52. 1943 (Marshall Is.); Smith, Sea Fishes S. Africa: 240, pl. 33, fig. 614. 1949 (Natal); de Beaufort, Fishes Indo-Aust. Arch., **9**: 128, fig. 24. 1951 (Indo-Pacific, not Hawaiian Is.); Harry, Atoll Res. Bull., 18: 150. 1953 (Raroia, Tuamotus).

Ctenodon ctenodon Fowler, Journ. Acad. Nat. Sci. Phila., **12**: 545. 1904 (Sumatra).

Ctenochaetus striatus Jordon & Seale (in part), Bull. Bur. Fish., **25**: 355. 1906 (Oceania, not Hawaiian Is.); Seale, Occ. Pap. B. P. Bishop Mus., **4**: 67. 1906 (Society Is., Cook Is., New Hebrides, Austral Is. and Solomon Is.); Evermann & Seale, Bull. Bur. Fish., **26**: 97. 1907 (Zamboanga, P. I.); Kendall & Radcliffe, Mem. Mus. Comp. Zoöl., **35**: 145. 1912 (Manga Reva); ?Jordan, Tanaka & Snyder, Cat. Fishes Japan: 634. 1913 (Misaki and Sagami, Japan); Fowler & Bean, Proc. U. S. Nat. Mus., **62**: 57. 1922 (Zamboanga, P. I.); Fowler, Proc. Acad. Nat. Sci. Phila., **75**: 42. 1923 (Madagascar); Herre, Philipp. Journ. Sci., **34**: 437, pl. 13, fig. 2. 1927 (Philippine Is.);—Field Mus. Nat. Hist. Zool. Ser., **21**: 246. 1936 (Tuamotus and Moorea); Schultz & Woods, U. S. Nat. Mus. Bull., 202: 620, pl. 61, fig. A, pl. 62, figs. A and B. 1953 (Marshall and Mariana Is.).

Teuthis striatus Barnard, Ann. S. Afric. Mus., **21**: 780. 1927 (Natal).

?*Ctenochaetus ctenodon* Whitley, Journ. Pan-Pacific Res. Inst., **3**: 12. 1928 (Santa Cruz Is.); Whitley & Colefax, Proc. Linn. Soc. New S. Wales, **63**: 294. 1938 (Nauru).

Diagnosis.—No black spot at axil of both dorsal and anal fins; teeth of upper jaw with 6 denticulations, of lower jaw 4, on lateral edge of expanded tips; membranes of pectoral fin hyaline; caudal fin lunate, caudal concavity 3.7 to 6 in standard length; dorsal soft rays usually 28 to 30.

Description.—Dorsal rays VIII, 27 to 31; anal rays III, 24 to 28; pectoral rays 16 or 17; anterior gill rakers 27 to 36; posterior gill rakers 29 to 42; scales from gill opening to posterior end of caudal peduncle spine 104 to 122.

Body depth 1.9 to 2.3, snout 4.2 to 4.8, pelvic fin 3.3 to 3.7, eighth dorsal spine 4.9 to 5.8, longest dorsal ray 3.6 to 4.4, third anal spine 5.4 to 6.7, longest anal ray 4.4 to 5.2, caudal concavity 3.7 to 6, all in standard length.

Margins and inner surfaces of lips smooth. Upper teeth with lateral edge of distal expanded end with 6 (rarely 5 or 7) denticulations. Lower teeth with 4 lateral denticulations at end (a small fifth one is sometimes evident).

Color in alcohol dark brown with numerous pale lengthwise slightly irregular lines on body (which are often faded and difficult to see); median fins brown; traces of about 5 lengthwise bands may at times be seen in the soft dorsal and anal fins; pectoral fin with rays light brownish (except uppermost principal ray which is edged in dark brown) and membranes hyaline; pelvic fins brown; young specimens may show a small black spot at the base of the last few dorsal rays. Color of juveniles dark brown with 8 to 12 pale longitudinal stripes on the body about ⅓ as

TABLE 3. COUNTS OF GILL RAKERS FROM THE FIRST GILL ARCH FOR THE SPECIES OF *Ctenochaetus*

Species and locality	Anterior series of gill rakers																					
	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42
striatus																							
Marshall, Mariana and Samoa Is.										1	1	3	5	4	4	3	1						
Philippine Is. and East Indies										3	5	6	3	1									
Egypt, Red Sea									1	2	1												
Mauritius											2	3	1	1									
strigosus																							
Hawaiian Is.										1	2	2	2	2	2								
Philippine Is.								2	1	1	1	1											
cyanoguttatus, sp. nov.																							
Gilbert and Phoenix Is.							1		1	1													
hawaiiensis, sp. nov.																							
Hawaii				2		2																	
magnus, sp. nov.																							
Malden, Jarvis, and Cocos I.									3	1													
tominiensis, sp. nov.																							
Celebes	1	3																					
binotatus, sp. nov.																							
Philippine Is.				1	2	1	2	2	2	1													

Species and locality	Posterior series of gill rakers																					
	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42
striatus																							
Marshall, Mariana and Samoa Is.												1		1		2	3	4	4	4	2		1
Philippine Is. and East Indies										1		1	1	2	3	2	4	3	1				
Egypt, Red Sea										1	2		1										
Mauritius													1	2	1	2	1						
strigosus																							
Hawaiian Is.											1	4	4	2									
Philippine Is.									1	3	1	1											
cyanoguttatus, sp. nov.																							
Gilbert and Phoenix Is.															1	1		1					
hawaiiensis, sp. nov.																							
Hawaii						1		2	1														
magnus, sp. nov.																							
Malden, Jarvis, and Cocos I.																1	3						
tominiensis, sp. nov.																							
Celebes	4																						
binotatus, sp. nov.																							
Philippine Is.			1		4	1	3	2															

broad as intermediate brown areas, which angle slightly downward as they pass posteriorly. At a standard length from 50 to 65 mm. the number of pale lines is suddenly doubled by the appearance of thin whitish lines in the center of the brown intermediate areas; these narrow lines become as broad as the pale lines of the first set, and more lines are added above and below, especially above, until the adult number of about 30 to 40 is attained. The ones added above the original set tend to angle upwards.

Color in life dark olive brown with blue lengthwise lines on the body and small orange spots on the head and nuchal region; soft dorsal and anal fins with about 5 lengthwise dark bluish lines; pectoral fin yellowish.

Juvenile specimens from the Gilbert Islands of about 40 mm. length were observed with the first set of pale lines red in color, narrow light bluish lines in the intermediate brown areas, and red tips to the lobes of the caudal fin. It is this color pattern that Herre (1927) probably tried to show in his Figure 2 of Plate 13 and which led him into the mistaken and persistent contention that this juvenile form was a different species. The error is an easy one to make in view of the variability in length at which adult coloration and configuration are assumed.

One specimen in the acronurus stage (transparent with silvery abdomen) was secured by night lighting at anchorage at Sydney Island in the Phoenix Islands by personnel of the Pacific Oceanic Fishery Investigations. This specimen, USNM No. 163616 and 32 mm. in standard length, is shown in Plate I, D. It is interesting to note that there is already a change-over taking place from larval dentition to adult-type dentition even though there is no evidence of any other bodily transformation to adult form. This change in dentition may be seen in only a slightly more advanced state in the transforming specimen of Plate I, E, from which the three teeth in Text-fig. 3 B to D were drawn. These teeth were all taken from the upper jaw of this specimen and show early stages in the transformation of tooth form. The inward bending of the expanded tips, already apparent in C, can only be seen in a side view of the teeth. The tooth drawn in A of Text-fig. 3 was taken from the *strigosus* acronurus of Plate I, D. All of its teeth are essentially alike; there are about 18 in each jaw. Probably this specimen is in an earlier stage of development than the *striatus* acronurus of Plate I, D. It is suspected that the teeth of *striatus* younger than the latter will bear 3 denticulations like the *strigosus* acronurus. The *striatus* acronurus has about 18 upper teeth and 16 lower teeth with more apparent just beneath the gums at the ends of the jaws. The lateral teeth are more adult-like in form than more medially-located teeth. The most striking thing about the dentition was the finding, upon dissection, of good-sized adult-type teeth imbedded in the soft tissue within the bones of the jaws. Five were found from one side of the upper jaw just above the upper teeth and six from one side of the lower jaw just below the lower teeth. Only the most medial of these imbedded teeth showed a departure from the *Ctenochaetus* pattern, for not all of the denticulations of this tooth were restricted to the lateral side. Subsequent dissection of the premaxillary and dentary bones of juvenile and adult specimens of *C. striatus* and other *Ctenochaetus* of all sizes showed these teeth to be present, in greater number and larger size in larger specimens, and it is believed that they function as replacement teeth. Specimens of species of *Acanthurus,* including acronurus and postacronurus forms, were examined, and adult-type teeth characteristic of this genus were also found imbedded in the bones of the jaws.

Remarks.—C. striatus is an exceedingly abundant and widespread species, probably occuring in the entire tropical Indo-Pacific region except the Hawaiian Islands. Jordan, Tanaka & Snyder (1913) record what is probably this species from Japan. J. L. B. Smith in a letter states that it appears to be the most abundant acanthurid on the reefs of tropical east Africa. At Onotoa Atoll in the Gilbert Islands, after two months of collecting, I found it was the dominant fish on a weight basis among all the reef fishes taken. It was very common on the benched reef slope (Cloud, 1952) of the windward reef of the atoll and about coral heads in the lagoon.

In the Red Sea *striatus* seems to have a lower gill raker count (Table 3) and also a greater percentage of specimens with 17 pectoral rays than 16 (Table 2). More material is needed from the Indian Ocean and Arabian Sea to clarify these and other differences.

Better established is the demarcation on the basis of dorsal and anal fin ray counts (Table 1) between populations in the Philippines and East Indies and the rest of Oceania. Also from the examination of fin ray counts, southeast Oceania (i.e., Society Islands and Tuamotus) may represent a differentiated population.

The largest specimen seen by me was 195 mm. in standard length. It was taken in the Philippines.

Taxonomic Discussion. — In all probability *Acanthurus argenteus* Quoy & Gaimard is a transforming *Ctenochaetus striatus.* These authors suspected that it was the young of some species they had not collected, but the nature of

the striped color pattern and the high fin ray counts leave little doubt that it is the same as *striatus*. I retain the name *striatus* because it has been commonly used, whereas *argenteus* has been recognized as a name applying to an immediate postacronurus form (the name referring to the silvery coloration of the abdomen) and has never been applied to any adult surgeon fish.

The consideration of *Acanthurus flavoguttatus* Kittlitz (*A. Ketlitzii* Cuvier & Valenciennes) as a synonym of *C. striatus* is based on the Kittlitz figure which is greenish with yellow spots on the head and yellow lines on the body. Lack of reference to dentition and the peculiar fin ray counts account for the uncertainty in my decision. The type of *flavoguttatus* was not located.

Acanthurus ctenodon Cuvier & Valenciennes was referred to synonymy by Kner (1865) and by Günther (1873).

Much confusion has resulted from the frequent use of the name *strigosus* for the species *striatus*. This seems to stem from Günther (1873) who considered *striatus* the young of *strigosus* and in the overlooking by Gill (1884) of the Quoy & Gaimard species altogether in his erecting of the genus *Ctenochaetus*. Jordan & Evermann (1905) perpetuated the error by copying Günther's plate of *striatus* and using it to represent the common Hawaiian species which, in reality, is *strigosus*. Fowler (1928) did not help by referring *striatus* to the synonymy of *Acanthurus lineatus*.

Herre's (1927) separation of *strigosus* and *striatus* was based on specimens not exceeding 60 mm. in length and is erroneous.

Ctenochaetus strigosus (Bennett)

Plate II, Fig. 1; Text-fig. 1 B; Plate I, A-C; Text-fig. 3 A

Acanthurus strigosus Bennett, Zool. Journ., **4**: 41. 1828 (type locality, Hawaiian Is.); Cuvier & Valenciennes (in part?), Hist. Nat. Poiss., **10**: 243. 1835 (Hawaiian Is., probably not New Guinea); Günther (in part?), Cat. Fish. Brit. Mus. **3**: 342. 1861 (Hawaiian Is., probably not New Guinea).

Acanthurus (*Etenodon*) *strigosus* Steindachner, Denkschr. Akad. Wiss., **70**: 495. 1901 (Honolulu).

Ctenochaetus strigosus Jenkins, Bull. U. S. Fish. Comm., **22**: 480. 1903 (Honolulu); Snyder, Bull. U. S. Fish. Comm., **22**: 534. 1904 (Honolulu); Fowler & Ball (in part), B. P. Bishop Mus. Bull., 26: 19. 1925 (Laysan, French Frigate Shoals and Johnston I., but not Wake I.); Fowler (in part), Mem. B. P. Bishop Mus., **10**: 274. 1928 (only Hawaiian Is.); Fowler & Bean (in part), U. S. Nat. Mus. Bull., 100, **8**: 200. 1929 (Philippines and East Indies); Fowler, Mem. B. P. Bishop Mus., **11**: 344. 1931 (Honolulu);—Acad. Nat. Sci. Phila. Mon., 2: 233 (only). 1938 (Honolulu);—Proc. Acad. Nat. Sci. Phila., **93**: 257. 1941 (Honolulu); Whitley, Proc. Roy. Soc. New S. Wales: 23. 1954 (Great Barrier Reef, Australia).

Ctenochaetus striatus Jordan & Evermann, Bull. U. S. Fish. Comm., **23**: 398, (Fig. 174 after Günther, not *strigosus*), 1905 (Hawaiian Is.); Jordan & Jordan, Mem. Carnegie Mus., **10**: 66. 1922 (Hawaiian Is.).

Ctenochaetus flavicauda Fowler, Acad. Nat. Sci. Phila. Mon., 2: 104, pl. 10, fig. 24. 1938 (Takaroa, Tuamotus);—Mem. B. P. Bishop Mus., **12**: 104. 1949 (Takaroa, Tuamotus).

Ctenochaetus sp. Harry, Atoll Res. Bull., **18**: 151. 1953 (Raroia, Tuamotus).

Diagnosis.—No black spot at axil of dorsal or anal fin; teeth of upper jaw with 5 denticulations, of lower jaw 3, on lateral edge of expanded tips; membranes of pectoral fin hyaline; caudal fin moderately concave, caudal concavity 5.7 to 10 in standard length; dorsal soft rays usually 26 or 27.

Description.—Dorsal rays VIII, 25 to 28; anal rays III, 21 to 25; pectoral rays 16 (rarely 15); anterior gill rakers 27 to 34; posterior gill rakers 28 to 33; scales from gill opening to posterior end of caudal peduncle spine 85 to 96.

Body depth 1.7 to 2, snout 4.3 to 4.8, pelvic fin 3.1 to 3.4, eighth dorsal spine 4.8 to 5.5, longest dorsal ray 3.8 to 4.2, third anal spine 5.3 to 6.5, longest anal ray 4.4 to 4.7, caudal concavity 6 to 10, all in standard length.

Margins and inner surfaces of lips smooth; upper teeth with 5 denticulations (occasional teeth from Philippine specimens will show tiny 6th denticulation); lower teeth with 3 denticulations (including tip).

Color in alcohol brown with about 35 narrow pale bluish longitudinal lines (about ¼ as broad as the alternate brown bands) on the body which angle upwards on the basal part of the dorsal fin and downwards on the basal part of the anal; small pale spots on the head (and anteriorly on the body in Philippine specimens); a pale ring, broader posteriorly, around the eye in Hawaiian and Tuamotu specimens, restricted to the posterior edge of the eye in Johnston Island material, and absent in Philippine specimens; median fins brown (except caudal fin of Tuamotu specimens which is abruptly pale, white in life); pectoral fin pale except edge of uppermost principal ray which is almost black; pelvic fins brown.

Color in life from a 35 mm. kodachrome transparency of a Hawaiian specimen brown with narrow pale blue longitudinal lines on the body and basally on dorsal and anal fins; purplish region on chin; blue spots on head; yellow ring around eye; caudal fin brown; pectoral fin rays brownish, membranes orange-yellow; pelvic fins brown.

Hawaiian specimens in life show considerable variation in ground color. Some, especially in a light-colored environment (as areas of high coral cover), become a light tan in color. Others may be dark brown. The ground color of the species at Raroia, Tuamotus, was observed by Harry (1953) to be black.

Remarks.—Along with the color differences noted above, and meristic data, differences in the shape of the caudal fin may be seen among the specimens from the Philippines and East Indies, Hawaiian Islands, Johnston Island, Tuamotus and Mauritius which might form the basis for the recognition of subspecies when more material is available and the range of the species more completely known. The single specimen from Mauritius has the least concave caudal fin, the caudal concavity being contained in the standard length about 20 times. The caudal concavity of specimens from the Philippines and the Hawaiian Islands is 7 to 10. Johnston Island specimens have a caudal concavity which ranges from 5.7 to 9 in the standard length; Tuamotu specimens have the most lunate caudal fins, the caudal concavity contained about 5.7 times in the standard length.

Harry (1953) reported acute pain and swelling in the hand and arm when cut on the hand by the caudal spine of this species in Raroia; the pain did not subside until the second day and persisted for a week. He added that the only other surgeon fish he encountered producing such effects was *C. striatus* (recorded as *strigosus*), though pain from this species lasted only 3 to 4 hours and was not so intense. I tested the poisonous qualities of the caudal spine of Hawaiian *strigosus* by gingerly inserting the tip of the spine into my palm; a stinging sensation was soon experienced, and the experiment was carried no further.

This species is extremely abundant in the Hawaiian Islands where it is known by the local name Kole. Elsewhere, except perhaps Johnston Island, it is not at all common. Only 13 specimens from the Philippines, Moluccas and Celebes were found at the U. S. National Museum among the vast collections made by the *Albatross* Philippine Expedition (1907-1910); only four are known from the Tuamotus, and one from Mauritius. The single specimen from the Great Barrier Reef was identified by Whitley with the aid of a manuscript key such as appears in this paper and was recorded from Australia by him (1954).

Although more collecting in the Indo-Pacific region may reveal new localities for *strigosus*, it is believed that it is not in continuous distribution throughout its range. The differentiation which has taken place in five of its known localities supports this contention. The great abundance of this species in Hawaii stands in sharp contrast to its apparent absence from much of the Indo-Pacific and its scarcity in the few areas where it does occur. This may be associated with the absence of *striatus* from the Hawaiian Islands.

The acronurus specimen (Plate I, A), U.S.-N.M. 118040 and 27 mm. in standard length, was taken at night from the steamer *Albatross* at the surface at Diamond Head Light, Oahu, Hawaiian Islands, on May 6, 1902. The transforming specimen of Plate I, B (University of Hawaii No. 1877) was collected by W. A. Gosline on the reef at Diamond Head, Oahu, May 16, 1950. It is 28 mm. in standard length. The recently transformed juvenile (Plate I, C), U.S.N.M. 167199, was taken by the author at a depth of 40 feet, Waikiki, Oahu, on June 4, 1952. As no small juveniles have been seen in tidepools or very shallow water, it is suspected that the acronurus transforms to the juvenile stage at a moderate depth on the reef. Postacronurus and juvenile *striatus*, on the other hand, occur in immense numbers in tidepools and protected shallow-water areas.

The largest specimen of *strigosus* seen by me, 139 mm. in standard length, was collected by the *Albatross* Philippine Expedition at Luzon.

The food habits of this species are discussed in the general section on the genus.

Ctenochaetus cyanoguttatus, sp. nov.

Text-fig. 1 C

?*Acanthurus guttatus* Kittlitz (not of Bloch & Schneider), Mus. Senckenb., **1**: 193, pl. 13, fig. 4. 1834 (Luganor I.).

?*Acanthurus marginatus* Cuvier & Valenciennes, Hist. Nat. Poiss., **10**: 221. 1935 (new name for *Acanthurus guttatus* Kittlitz); Günther, Cat. Fish. Brit. Mus., **3**: 333.1861 (Luganor).

?*Acanthurus ctenodon* Var. *b* Playfair, Fishes of Zanzibar, 57. 1866. (Zanzibar).

Ctenochaetus strigosus Snodgrass & Heller, Proc. Washington Acad. Sci., **6**: 402. 1905 (Cocos I., Costa Rica); Schultz (in part), U. S. Nat. Mus. Bull., 180: 161. 1943 (Hull I.).

Ctenochaetus sp. Hiyama, Poisonous Fishes South Seas: 92, pl. 19, fig. 53. 1943 (Marshall Is.).

Holotype.—U.S.N.M. No. 167178, Aunteuma Island, Onotoa Atoll, Gilbert Islands, lee side, depth of water about 5 feet in poorly-defined surge channel region, August 1, 1951, spear, John E. Randall, 170.5 mm. in standard length.

Paratypes.—U.S.N.M. No. 167179, same data as holotype except date, August 11, 1951, 166 mm.; U.S.N.M. No. 115152, Hull Island, reef, July 13, 1939, Leonard P. Schultz, 157 mm.; Stanford Mus. No. 12280, Cocos Island, Costa Rica, Hopkins-Stanford Galapagos Expedition, 1898-1899. (Precise proportional measurements and scale and gill raker counts were not made on the Cocos Island specimen).

Diagnosis.—No black spot at axil of dorsal or anal fin; teeth of upper jaw with 4 denticulations, of lower jaw 3, on lateral edge of expanded tips; membranes of pectoral fin dark brown; caudal concavity 5.8 to 6.2 in standard length; dorsal soft rays 27 or 28.

Description.—Dorsal rays VIII, 27 (27 to 28); anal rays III, 25; pectoral rays 17 (16 to 17); anterior gill rakers 28 (26 to 29); posterior gill rakers 35 (34 to 37); scales from gill opening to posterior end of caudal peduncle spine 95 (94-104); upper teeth 44, lower teeth 60.

Depth of body 1.85 (1.94 to 1.95), length of head 3.48 (3.27 to 3.61), length of snout 4.34 (4.36 to 4.74), length of pectoral fin 3.10 (3.02 to 3.13), length of pelvic fin 2.94 (2.91), length of 8th dorsal spine 5.09 (5.24 to 5.27), length of longest dorsal ray 5.14 (4.62 to 4.89), length of third anal spine 5.89 (5.81 to 6.15), length of longest anal ray 5.01 (5.42 to 5.44), caudal concavity 6.20 (5.81 to 5.93), all in standard length. Greatest diameter of eye 4.41 (4.26 to 4.40), width of inter-orbital space 3.06 (2.87 to 3.1), least depth of caudal peduncle 2.27 (2.19 to 2.34), length of caudal peduncle spine 2.90 (2.42 to 2.67), distance from base of upper lip to distal ends of upper teeth 5.1 (4.9 to 5.1), all in length of head.

Inner surfaces of lips and margin of upper lip smooth; margin of lower lip, especially posteriorly, papillate. Upper teeth with 4 (rarely 3) denticulations (the one at the tip being longest); lower teeth with 3 denticulations (counting tip).

Color in alcohol dark brown with numerous small pale spots on the body and pectoral fin (these spots are faint and difficult to see even in freshly preserved fish); all fins brown; dorsal and anal fins with about 9 or 10 longitudinal dark brown lines (fewer anteriorly in these fins).

Color in life dark brown with head, body, and pectoral fins profusely covered with small bright blue spots, hence the name, *cyanoguttatus.*

Remarks.—This species was observed by me in its natural habitat on two occasions at Onotoa, Gilbert Islands. It was seen in small rapidly-moving schools in moderately rough water in broad shallow surge channels on the lee side of the atoll. It was a mode of life similar to that of *Acanthurus guttatus,* the latter species schooling in the rougher water of sharply-defined surge channels on the windward side of the atoll. The spotting of the body of both these species might be associated with the masses of small swirling air bubbles in the water that characterize the surf zone where they live.

Taxonomic Discussion.—*Acanthurus guttatus* Kittlitz was a brown fish with blue spots and 8 dorsal spines. Cuvier & Valenciennes, realizing that this could not be the *A. guttatus* of Bloch & Schneider, gave the species the name *marginatus.* It is possible that this species was *Ctenochaetus cyanoguttatus.* But no mention was made of the important item of dentition, although the teeth in Kittlitz' figure are drawn fairly long. The count of dorsal spines cannot be considered too diagnostic, for species of *Acanthurus* have been recorded with 8 instead of 9 dorsal spines because of the inconspicuous nature of the first spine. Even the blue spots are not unique, for adult *Acanthurus nigroris* Cuvier & Valenciennes (=*Hepatus atramentatus* Jordan & Evermann) at Wake Island had the usual blue lines broken up into spots, which gave this species an appearance much like *C. cyanoguttatus.* In view of this, and the fact that the Kittlitz specimen has not been located, describing the species as new seems in order. The Kittlitz specimen is not in the Senckenberg Museum. Günther (1861) stated that the type of *Acanthurus pyroferus* described by Kittlitz from the same publication was in the old St. Petersburg Museum. The specimen of *A. marginatus* may be there; I have been unable to complete correspondence on the matter.

The Cocos Island specimen of Snodgrass & Heller (1904) is referable to this species, although there is no record of the color in life and the spots which were probably present have now faded. G. S. Myers, who kindly loaned the specimen (SU 12280) to me, states that only one other of the four specimens collected from Cocos is now located at the Natural History Museum, Stanford University.

Ctenochaetus hawaiiensis, sp. nov.

Plate II, Fig. 2; Text-fig. 1 D

Ctenochaetus hawaiiensis Brock, Journ. Wildlife Man., **18**: 307. 1954 (nomen nudum; name used by Brock from a personal communication before species published).

Holotype.—U.S.N.M. No. 167180, entrance

to Keauhou Bay, Hawaii, Hawaiian Islands, depth of water about 12 feet, February 8, 1952, spear, John E. Randall, 197.5 mm. in standard length.

Paratypes.—U.S.N.M. No. 167181, Keahole Point, Hawaii, Hawaiian Islands, depth of water 40 feet, June 18, 1953, spear, Vernon E. Brock, 2 specimens, 204 and 209 mm.; Standford Mus. No. 47661, same data as other paratypes, 202.5 mm.

Diagnosis.—No black spot at axil of dorsal or anal fin; teeth of upper and lower jaws with 3 denticulations on lateral edge of expanded tips; membranes of pectoral fin dark brown; twice as many teeth in lower jaw as upper; caudal fin slightly emarginate to nearly truncate; dorsal soft rays 27 or 28.

Description.—Dorsal rays VIII, 28 (27 to 28); anal rays III, 25 (25 to 26); pectoral rays 16; anterior gill rakers 25 (23 to 25); posterior gill rakers 28 (25 to 27); scales from gill opening to posterior end of caudal peduncle spine 139 (115 to 131); upper teeth 26; lower teeth 56.

Depth of body 1.81 (1.82 to 1.84), length of head 3.00 (3.27 to 3.32), length of snout 3.66 (3.81 to 3.92), length of pectoral fin 3.05 (3.07 to 3.21), length of pelvic fin 3.87 (3.61 to 3.78), length of eighth dorsal spine 4.60 (4.50 to 4.80), length of longest dorsal ray 4.12 (4.18 to 4.21), length of third anal spine 6.49 (5.96 to 6.97), length of longest anal ray 4.49 (4.50 to 4.86), caudal concavity 18.8 (15.0 to 40.8), all in standard length. Greatest diameter of eye 4.86 (4.39 to 4.85), width of inter-orbital space 3.30 (2.97 to 3.14), least depth of caudal peduncle 2.30 (2.17 to 2.38), length of caudal peduncle spine 3.14 (2.87 to 3.75), distance from base of upper lip to distal ends of upper teeth 3.66 (3.15 to 3.64), all in length of head. The last-mentioned measurement, which might also be loosely termed the height of upper lip, is greater in this species than any other in the genus (this distance in other species is contained in the head length 4.5 to 6.6 times).

Margins of lips finely crenulate; distal one-fourth of inner surfaces of lips plicate, the ridges running perpendicular to the margin. Upper and lower teeth with 3 denticulations (including tips); lower teeth about twice as numerous as upper teeth.

Color in alcohol very dark brown with many narrow pale longitudinal lines faintly visible on head and body; (these lines tend to be less irregular than those on other species; the lines on the head are somewhat diagonal); all fins dark brown.

Color in life dark olive brown (appearing almost black underwater) with fine yellowish-gray lengthwise lines on the head and body.

Remarks.—This species is thus far known only from the island of Hawaii in the Hawaiian Islands, where it is common; it is named for this locality. Fishermen have told me that it is seen rarely at the island of Maui. It certainly does not seem to be present in the waters around the island of Oahu, which are well-collected.

I have observed *hawaiiensis* underwater on only four occasions, three times as a solitary fish and once as a group of three fish. Vernon E. Brock, Division of Fish and Game, Territory of Hawaii, has told me that he has observed the species in schools.

A smaller specimen (estimated 100 mm. in standard length), yellowish-brown in color, and possibly *hawaiiensis,* was sighted by me at a depth of 70 feet in Kealakekua Bay, Hawaii, but was not taken.

Ctenochaetus magnus, sp. nov.

Text-fig. 1 E

Ctenochaetus strigosus Fowler (in part), B. P. Bishop Mus. Bull., 38: 20. 1927 (Jarvis I.).

Holotype.—U.S.N.M. No. 163614, Malden Island (4° 03′ S., 154° 59′ W.), January 27, 1951, spear, Herbert Mann and Joseph E. King, Pacific Oceanic Fishery Investigations, 225 mm. in standard length.

Paratypes.—Stanford Mus. No. 47662, same data as holotype, 219 mm.; B. P. Bishop Mus. No. 4308, Jarvis Island (0° 23′ S., 160° 02′ W.), August 15, 1924, Whippoorwill Expedition, 205.5 mm., U.S.N.M. No. 163659, Cocos Island, Costa Rica, December 28, 1952, Bruce W. Halstead and Norman C. Bunker, 209 mm.

Diagnois.—No black spot at axil of dorsal or anal fin; teeth of upper and lower jaws with 3 denticulations on lateral edge of expanded tips; membranes of pectoral fin dark brown; 1.2 times as many teeth in lower jaw as upper jaw; caudal concavity 6 to 7 in standard length; dorsal soft rays 26 or 27.

Description.—Dorsal rays VIII, 27 (26 to 28); anal rays III, 25 (24 to 25); pectoral rays 17 (16 to 17); anterior gill rakers 28 (28 to 29); posterior gill rakers 36 (35 to 36); scales from gill opening to posterior end of caudal peduncle spine 159 (149 to 164); upper teeth 59; lower teeth 68.

Depth of body 2.14 (2.02 to 2.13), length of head 3.14 (3.32 to 3.48), length of snout 4.60 (4.18 to 4.76), length of pectoral fin 3.13 (3.05 to 3.17); length of pelvic fin 2.87 (2.98 to 3.16), length of eighth dorsal spine 5.00 (5.76 to 5.97), length of longest dorsal ray 5.92 (5.54 to 5.63), length of third anal spine 6.52 (6.26 to

6.74), length of longest anal ray 6.00 (5.02 to 6.44), caudal concavity 5.63 (6.04 to 7.45), all in standard length. Greatest diameter of eye 5.07 (4.50 to 4.88), width of inter-orbital space 2.87 (3.00 to 3.07), least depth of caudal peduncle 2.27 (2.22 to 2.47), length of caudal peduncle spine 2.36 (2.33 to 2.57), distance from base of upper lip to distal ends of upper teeth 5.28 (4.5 to 5.3), all in length of head.

Margins and inner surfaces of lips smooth. Upper and lower teeth with 3 denticulations (including tips).

In alcohol the color of this species is uniform dark brown. The only record of color in life which I have is that given by Fowler (1927) for the Jarvis Island specimen. He states that the body including the pectorals was covered all over with fine blue-gray dots.

Remarks.—Thus far *Ctenochaetus striatus* (and *C. strigosus* as well) is not known from Malden, Jarvis and Cocos Islands, in spite of its common occurrence elsewhere in the tropical Pacific. These islands are the sole known localities for *C. magnus.*

Assuming that *Ctenochaetus* is Indo-Pacific in origin, which seems reasonable in view of the distribution of the species of this genus and the Acanthuridae in general, one must explain how *magnus* (and *cyanoguttatus*) crossed the East Pacific barrier (Ekman, 1953). Malden Island and especially Jarvis Island are near the counter-equatorial current which could conceivably carry fish larvae the great distance to Cocos Island (possibly via the Galapagos Islands). Herre (1940) discusses this mode of transport.

This species is named *magnus* in reference to its large size.

Ctenochaetus tominiensis, sp. nov.

Text-fig. 1 F

Ctenochaetus strigosus Fowler & Bean (in part), U. S. Nat. Mus. Bull., 100, **8**: 200. 1929 (Gulf of Tomini, Celebes, only).

Holotype.—U.S.N.M. No. 136112, Sadaa Island, Gulf of Tomini, Celebes, November 17, 1909, dynamite, *Albatross* Philippine Expedition, 98 mm. in standard length.

Paratypes.—U.S.N.M. No. 136093, Buka Island, Gulf of Tomini, Celebes, November 20, 1909, dynamite, *Albatross* Philippine Expedition, 4 specimens, 74 to 100 mm.; Stanford Mus. No. 47663, same data as other paratypes, 87 mm.

Diagnosis.—A black spot at axil of both the dorsal and the anal fins; teeth of upper jaw with 3 denticulations on upper lateral edge of expanded tips; teeth of lower jaw with 3 denticulations; membranes of caudal fin, posterior parts of dorsal and anal fins and pectoral fins pale; margins of lips papillate; dorsal soft rays 24 or 25.

Description.—Dorsal rays VIII, 24 (25); anal rays III, 22 (22 to 23); pectoral rays 15, 16 (15 to 16); anterior gill rakers 21 (20 to 21); posterior gill rakers 20 (20); scales from gill opening to posterior end of caudal peduncle spine 91 (83 to 86); upper teeth 33; lower teeth 32.

Depth of body 2.04 (1.80 to 2.00), length of head 3.49 (3.22 to 3.37), length of snout 4.56 (4.40 to 4.65), length of pectoral fin 3.06 (2.69 to 3.03), length of pelvic fin 3.50 (3.28 to 3.59), length of eighth dorsal spine 6.12 (5.33 to 6.06), length of longest dorsal ray 3.50 (3.57 to 4.40), length of third anal spine 6.95 (5.87 to 6.90), length of longest anal ray 3.92 (4.00 to 4.11), caudal concavity 5.03 (4.35 to 6.52), all in standard length. Greatest diameter of eye 3.37 (3.17 to 3.53), width of inter-orbital space 2.92 (2.81 to 3.09), least depth of caudal peduncle 2.29 (2.23 to 2.44), length of caudal peduncle spine 2.55 (2.50 to 3.25), distance from base of upper lip to distal ends of upper teeth 6.20 (6.00 to 6.10), all in length of head.

Margins of lips papillate or crenulate, inner surfaces smooth. Upper teeth with distal half of the expanded ends smooth and blade-like and basal half divided into 3 (rarely 2) lateral denticulations; lower teeth with 3 (occasionally 4) denticulations (including tip).

Color in alcohol brown with a jet black spot at the base of the last few dorsal and anal fin rays, these spots extending slightly on to the caudal peduncle; caudal fin pale yellowish, gradually becoming brown basally, outer portions of the soft dorsal and anal fins pale yellowish, especially posteriorly, basally brown like body; outer portion of the brown part of these fins with about 3 to 5 narrow pale horizontal bands (difficult to see on some specimens) which become confluent with the pale distal region of the fins as they pass posteriorly; pectoral fins with rays brownish, membranes pale; pelvic fins brown, a little darker terminally.

The following color note from fresh specimens was taken from Fowler & Bean (1929); it was associated with this species by means of *Albatross* field numbers. "Brownish, dark in life, spotting of side of head indistinct, lower part slightly paler. Dorsal olive, with 5 or 6 bars, beginning as darker olive and on soft fin become cadmium or orange, fuse on fin posteriorly and terminally to form entire color; extreme fin edged narrowly black above; black blotch at axil. Anal like dorsal. Caudal fades whitish. Ventral blackish terminally, membranes hyaline

and scattered small, orange, basal spots, rays probably black in life."

Remarks.—Named *tominiensis* for the Gulf of Tomini, Celebes.

CTENOCHAETUS BINOTATUS, sp. nov.

Text-fig. 1 G

Ctenochaetus strigosus Fowler & Bean (in part), U. S. Nat. Mus. Bull., 100, **8**: 200. 1929 (Philippine Is. and East Indies).

Holotype.—U.S.N.M. No. 136125, Pagapas Bay, Luzon, Philippine Islands, February 20, 1909, dynamite, *Albatross* Philippine Expedition, 111 mm. in standard length.

Paratypes.—U.S.N.M. Nos. 136061, 136064, 136073, 136075 to 136080, 136083, 136095, 136099, 136102 to 136105, 136109, 136111, 136114, 136116, 136119, Philippines and Moluccas, January 20, 1908, to November 24, 1909, all taken with use of dynamite except one specimen from market at Cebu Island, *Albatross* Philippine Expedition, 27 specimens, 79 to 141 mm.; Stanford Mus. No. 27174, Jolo, Philippines, August 15, 1931, A. W. Herre, 2 specimens, 106 and 121 mm.

Diagnosis.—A black spot at axil of both the dorsal and anal fins; teeth of upper jaw with 6 denticulations, of lower jaw 3, on lateral edge of expanded tips; membranes of caudal fin and dorsal and anal fins dark brown; membranes of pectoral fin hyaline; margins of lips smooth; dorsal soft rays 24 to 27 (mostly 26).

Description.—Dorsal rays VIII, 25 (24 to 27), anal rays III, 23 (22 to 25), pectoral rays 16 (15 to 16), anterior gill rakers 29 (23 to 29), posterior gill rakers 24 (22 to 27), scales from gill opening to posterior end of caudal peduncle spine 102 (93 to 100), upper teeth 39, lower teeth 42.

Depth of body 1.93 (1.88 to 2.03), length of head 3.47 (3.29 to 3.79), length of snout 4.74 (4.77 to 5.47), length of pectoral fin 2.77 (2.86 to 3.10), length of pelvic fin 3.47 (3.44 to 3.78), length of eighth dorsal spine 5.84 (5.64 to 6.15), length of longest dorsal ray 3.77 (3.61 to 4.58), length of third anal spine 5.84 (5.81 to 6.21), length of longest anal ray 4.21 (4.20 to 4.74), caudal concavity 4.27 (4.51 to 6.15), all in standard length. Greatest diameter of eye 3.41 (3.12 to 3.91), width of inter-orbital space 2.91 (2.60 to 3.16), least depth of caudal peduncle 2.19 (1.97 to 2.32), length of caudal peduncle spine 2.74 (2.43 to 3.60), distance from base of upper lip to distal ends of upper teeth 6.4 (5.5 to 6.6), all in length of head.

Margins and inner surfaces of lips smooth. Upper teeth with expanded distal part divided into 6 approximately equal lateral denticulations; lower teeth with 3 denticulations (counting tip).

Color in alcohol brown with pale lengthwise lines on the body, (much as in *strigosus* except these lines are about twice as broad as the alternate darker brown lines); a prominent black spot at the base of the last few dorsal and anal fin rays, these spots extending narrowly on to the caudal peduncle; median fins brown; pectoral rays light brownish, the membranes hyaline; margin of uppermost principal pectoral ray dark brown; pelvic fins yellowish, lateral edges and ends of rays brownish.

There is no positive record of the color in life. Fowler & Bean (1929) give a color note for three specimens, one of which was identified as the type of *binotatus* by virtue of the *Albatross* field number; however, one of the remaining specimens (the other was not located) proved to be *C. striatus*. The color description is as follows: "Fine stripes of light bluish on the shoulder at and below the pectoral base and body posteriorly greenish. On dorsal 5 or 6 densely greenish stripes, similarly on anal. First pectoral ray very dark, center of fin yellow. Ventrals like body. Some examples with fine lines on body very deep violet." Both the type of *binotatus* and the one specimen of *striatus* faintly show in the preserved state the narrow lengthwise lines posteriorly on the body as well as the shoulder and pectoral region. It seems odd that the posterior markings would not be apparent in life.

Remarks.—Named *binotatus* for the two black marks, one at the axil of the soft dorsal fin and the other at the axil of the anal fin.

A recently-transformed 35 mm. specimen of *Ctenochaetus* (U.S.N.M. No. 167177), collected in the lagoon at Onotoa Atoll, Gilbert Islands, by the author was noted to have a brilliant yellow caudal peduncle and caudal fin and stood out in sharp contrast to the young of *C. striatus* picked up at the same time. Examination of the preserved specimen some months later revealed the last few rays of the soft dorsal and anal fins to be colorless and a small spot to be present in each fin at the base of these rays; small dark brown spots were observed on the head and anteriorly on the body. Meristic data are as follows: D VIII, 27; A III, 25; pectoral rays 15; anterior gill rakers 27; posterior gill rakers 27, upper teeth 22, lower teeth 22. The upper teeth bear 6 or 7 denticulations (usually 7) and the lower teeth 3 or 4 (mostly 4). Of the known species of *Ctenochaetus*, this specimen would best receive the label of *binotatus*; however, there are some obvious differences such as coloration and structure of the teeth. Since no specimens of *binotatus* below 79 mm. in stan-

dard length are available for comparison and none of any size from Gilbert Islands (if the species occurs there), the differences observed may be due to the juvenile nature of the specimen or the geographical separation of the Gilbert Islands from the Philippines and East Indies. It is certain that the specimen is not *Ctenochaetus flavicauda* Fowler, for the latter is a variant of C. *strigosus*, and probably had a white tail in life instead of a yellow one.

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[2] See synonymy for taxonomic references.

EXPLANATION OF THE PLATES

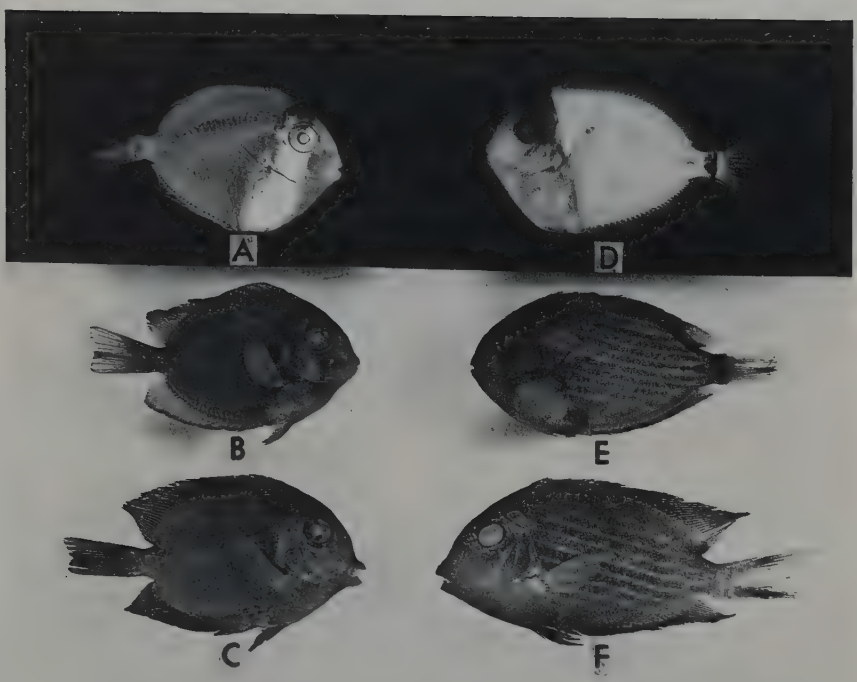
Plate I

Acronurus, postacronurus, and juvenile stages of *Ctenochaetus*. A to C. *strigosus*. D to F. *striatus*. Natural size.

Plate II

Fig. 1. *Ctenochaetus strigosus* (Bennett). 95 mm. specimen from the Hawaiian Islands drawn by Miss Marion Adachi.

Fig. 2. *Ctenochaetus hawaiiensis*, sp. nov. Drawing of holotype by Miss Marion Adachi.



A REVISION OF THE SURGEON FISH GENUS CTENOCHAETUS, FAMILY ACANTHURIDAE, WITH DESCRIPTIONS OF FIVE NEW SPECIES

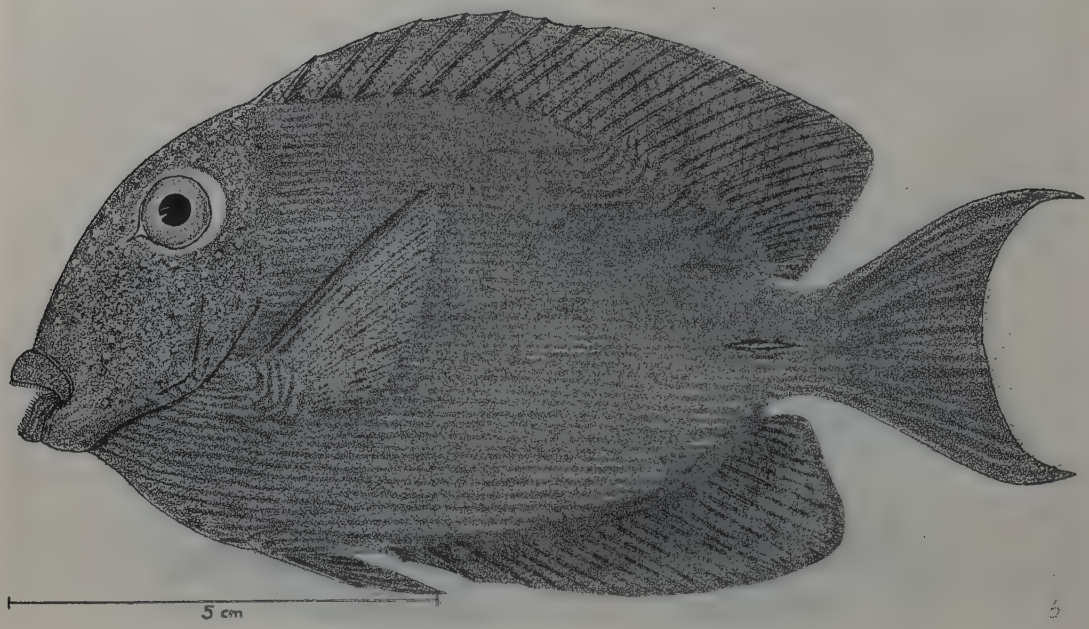


FIG. 1

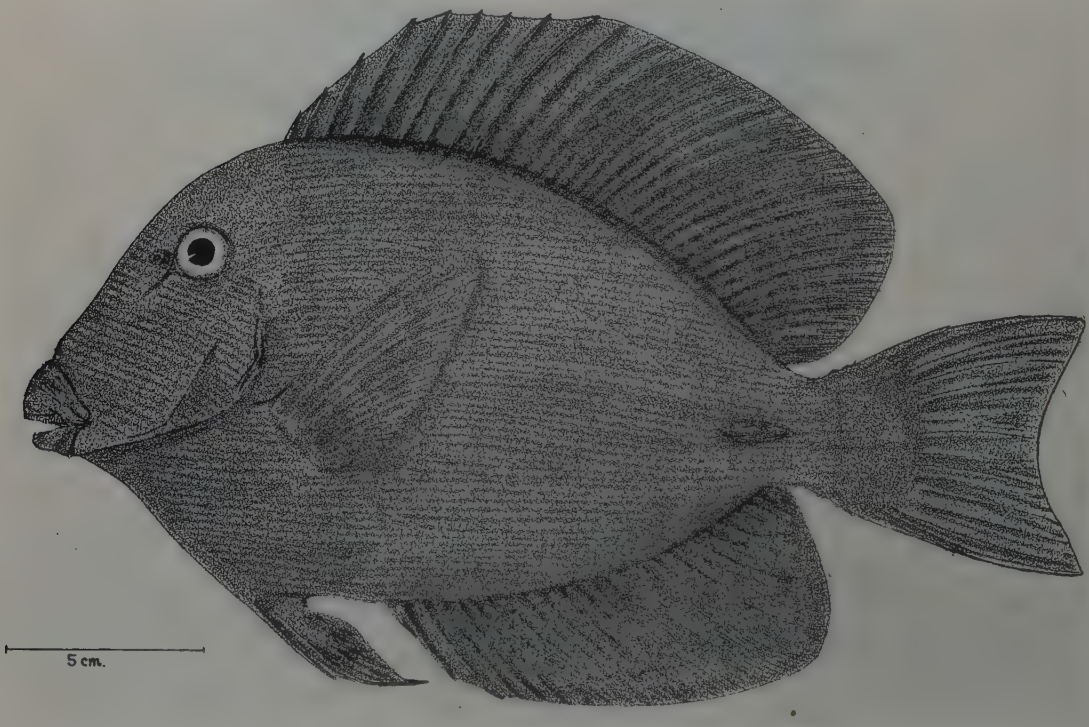


FIG. 2

A REVISION OF THE SURGEON FISH GENUS CTENOCHAETUS, FAMILY ACANTHURIDAE, WITH DESCRIPTIONS OF FIVE NEW SPECIES

16

Imaginal Behavior of a Trinidad Butterfly, *Heliconius erato hydara* Hewitson, with Special Reference to the Social Use of Color[1]

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(Plates I-III; Text-figures 1 & 2)

[This paper is one of a series emanating from the tropical Field Station of the New York Zoological Society, at Simla, Arima Valley, Trinidad, British West Indies. This station was founded in 1950 by the Zoological Society's Department of Tropical Research, under the direction of Dr. William Beebe. It comprises 200 acres in the middle of the Northern Range, which includes large stretches of undisturbed government forest reserves. The laboratory of the station is intended for research in tropical ecology and in animal behavior. The altitude of the research area is 500 to 1,800 feet, with an annual rainfall of more than 100 inches.

[For further ecological details of meteorology and biotic zones see "Introduction to the Ecology of the Arima Valley, Trinidad, B.W.I.," William Beebe. (Zoologica, 1952, Vol. 37, No. 13, pp. 157-184.)].

CONTENTS

I. INTRODUCTION

Among the most interesting of neotropical insects are the predominantly red and black butterflies occurring in the genus *Heliconius*. Because of the great variety of patterns found in closely related or identical species, the group poses a famous problem in systematics. All of these butterflies are apparently strongly aposematic, and with their basic coloring of scarlet on black, they are considered a classic example of warning coloration. They also serve as one of the most often quoted illustrations of Müllerian mimicry, since the highly variable members of two distinct sections of the genus show many closely similar pairs of forms.

Their general ecology has long been familiar to collectors in the damper parts of the new world tropics. The butterflies are usually common in the various types of rain, montane rain, seasonal and swamp forests. They rarely occur in the depths of the forest itself, however, preferring its edges, glades, clearings, trails and roadsides. They also are typical inhabitants of well-grown open second growth, and of lands devoted to such crops as citrus, cocoa and bananas, provided only that woodland is in the vicinity and that the crops are not too cleanly cultivated.

Except for records of roosting aggregations, it appears that nothing has been published on the social behavior, including the courtship, of *Heliconius*. The purpose of the present paper is to describe and analyze this behavior in the common Trinidad form, *H. erato hydara* Hewitson, with special emphasis on the function of color in in-

[1]Contribution No. 963, Department of Tropical Research, New York Zoological Society.

traspecific relations. Because of the color variability in other localities and in related sympatric species, the possible role of this characteristic as a social releaser is considered to be of special interest from an evolutionary point of view. This is particularly so because one function of the garish colors is held by many, including the writer, to be aposematic.

In contrast to some continental forms (see especially Beebe, 1955) the Trinidad subspecies is little variable in color or pattern, being black with an unbroken red band running transversely across the middle of the forewing. Except for minor differences in the red, yellow and white dots and bars of head, thorax and basal underwings, macroscopic variation is confined to the irregularities of the margins of the red forewing bands, and to their exact width. A rare local variant has a single tiny red spot, less than 1 mm. across, near the anterior margin of the hindwing. The subspecies, as at present defined, ranges across northern South America from Panama to Trinidad. The species in the broadest sense apparently flies throughout the neotropics from Guatemala south, exclusive of the West Indies.

Because of the emphasis in the present study on the function of color, it was essential to conduct basic experiments on the existence and extent of color perception in this species. These results are included in the present report as prerequisites to the work on social behavior. However, a thorough study of spectral limits, the division of the spectrum into hues, and the precise boundaries between innate and learned aspects of feeding behavior must await further work. A basic remaining requirement is the making of electroretinograms.

Also only briefly treated in the present paper are the three or more odors so far detected in this species, as well as analytical work on roosting behavior.

It is a pleasure to thank the Research Laboratories of the Interchemical Corporation, New York, for contributing spectrophotometric curves of the numerous color samples used in the experiments, and Mr. Ernest C. Crocker, of the Flavor Laboratory of Arthur D. Little, Inc., Cambridge, for his report on the odors of *Heliconius* scent organs.

Deep appreciation goes to Dr. William Beebe, who inaugurated and took part in many of the observations recorded in the following pages. Hearty thanks also go to all members of the Department staff, Dr. Beebe, Mr. Henry Fleming, Miss Rosemary Kenedy and Dr. Richard Tashian, for their help in assembling and rearing specimens, for witnessing key experiments and for many most helpful suggestions.

II. Historical Review

Butterfly behavior has rarely been systematically observed, much less analyzed by experimental means. A principal exception is a study of the satyrid, *Eumenis semele* (Tinbergen *et al.*, 1941). In this contribution, the feeding and courtship behavior were described, and the respective roles of odor, movement, form and color as feeding and social releasers investigated.

Ilse's pioneer work on butterfly color vision and behavior is also outstanding (1928, 1932.1, 1932.2, 1937). She established beyond question the fact of butterfly color perception through their responses to artificial flowers. The models were made from series of standard gray and colored papers. Differences among the vanessids. pierids and papilionids in innate color preferences were found to occur, and foundations laid for determination of the number and boundaries of the hues distinguished.

Eltringham (1919, 1933) and Eltringham and Ford (Ford, 1945) performed experiments which produced positive responses in *Argynnis euphrosyne* to dyed specimens which had been previously dried and bleached, and to photographic models, painted to resemble living butterflies in color. Their results also indicated that these butterflies respond to color irrespective of motion or odor. However, the spectrophotometric reflectances of the paints and dyes used were not known, nor was hue completely separated from either brightness or pattern. In none of the above studies was the possible role of the ultraviolet investigated.

Similar experiments were carried out by Ilse (1937), by Eggers (1938.1, 1938.2) and by Petersen, Törnblom & Bodin (1952), all of whom worked with pierids. The latter contribution, based on responses of wild polymorphic species to dried specimens and to models, makes a needed beginning on the vital problem of visual releasers in polymorphic butterflies. The role of the low ultraviolet reflectance of white pierid wings remains uncertain (see also Crane, 1954, p. 108 and ref.).

Records of butterfly courtships, mostly descriptions of necessarily incomplete observations in the field, are scattered through the literature, particularly in the field accounts of the older naturalists. Carpenter (1935) gives a general discussion and a good bibliography. Courtships in the heliconines, however, have apparently been hitherto unknown. The only reference to mating behavior appears to be that of Edwards (1884) who reported on males of *H. charithonius* gathering on the chrysalids of females and mating with them on emergence. Territoriality is suggested by Seitz (1913, p. 377) who observed

heliconine males "promenading" up and down individual beats in the forest, day after day.

The emission of scent by butterflies, apparently sometimes as sexual attractants and sometimes for protection against predators, has been described from time to time. Notable are Longstaff's (1912) observations, which include descriptions of the strong, witch-hazel-like odor which some *Heliconius* emit when captured (pp. 492, 503-504). Poulton (1925, 1931.2), Longfield (1926) and Collenette (1929) added more details and opinions on the scents of heliconiids, including the subject of this paper. Müller's (1878) descriptions and figures of scent glands and scales in danaids and heliconines are classsics; translations have been published by Longstaff as appendices (*loc. cit.*). Eltringham (1925, 1926) published further studies of both the glands and their odors in *H. erato hydara,* as well as in the genera *Coloenis, Dione* and *Eueides;* these three genera included species now referred to *Dryas, Agraulis* and *Heliconius.* Finally Barth (1953) discussed the form and function of the scent organs of male Lepidoptera from a more generalized and comparative point of view.

Gregarious roosting habits have been reported for a number of butterflies, including representatives of the danaids, heliconiids, ithomiids and nymphalids (e.g. Beebe, 1918, pp. 203 ff.; Myers, 1930; Jones, 1930, 1931; Poulton, 1931.1, 1931.2; Poulton and others, 1933; Guppy, 1932 and Carpenter, 1932). The only experimental work has been that of Jones (*loc. cit.*) who determined that *H. charithonius* in Florida was attracted on successive nights by a general locality recognition of some kind, rather than to special twigs or branches. Hence the attraction was not to an odor left on particular resting places.

Gatherings of various butterfly species on damp sand or around mudholes are also well known (e.g. Collenette & Talbot, 1929), as are the winter aggregations of monarch butterflies, *Danaus plexippus,* as mentioned in, for example, Swain (1948, p. 109). Records of butterfly migrations in all part of the world are exceedingly numerous, the most intensive study having been made by Williams (1930). No migrations of heliconines appear to have been reported, except the occasional flights, small and moderately large, reported at Portachuelo Pass, Rancho Grande, in Venezuela by Beebe (1950).

Food plant selection and the general egg-laying habits of female butterflies have received the widest notice, chiefly because of the frequent economic importance of the subject. Seitz's (1913, p. 377) observations on *Heliconius* seem to be the only field notes for that genus, although the caterpillars are fairly well known. He observed these butterflies near Belém laying eggs on *Passiflora,* usually around noon; they apparently chose high-climbing vines.

A paper on the spectral composition of certain butterfly colors (Crane, 1954) includes an analysis of the red band in *H. erato* which was a prerequisite to the investigation of the band's function in intraspecific relations. It proved to have a minimum reflectance from the ultraviolet through the green, low in the yellow and high in both the orange and red.

III. Materials and Methods

Two circumstances permitted the undertaking of the present study. Of first importance was our location in a permanent tropical field station in an area where *Heliconius erato* is common. This contribution is the result of observations and experiments extending over parts of five years. The second factor was our maintenance of two large insectaries for the study of captive butterflies.

The construction and operation of these flying cages have been described elsewhere (Crane & Fleming, 1953). Here it need only be said that the structures measure 12′ × 18′ and 24′ × 36′, respectively, and are built of fine wire mesh on a wooden framework. Being floorless, they are planted with a profusion of herbs, shrubs and vines approximating natural conditions. Ample sun, shade and wind protection are essential as well as the maintenance of high humidity. Butterflies caught in the field are placed in envelopes, kept cool and released in the insectaries as soon as possible.

Heliconius erato proved to be the most amenable to life in the insectaries of numerous species tested. Once these large cages have been "seasoned," presumably through clinging species odors, newcomers settle in at once, feeding and successfully avoiding the wire within minutes of their release. Reared specimens are equally adaptable.

The favorite local food flower of these butterflies is *Lantana camera* Linnaeus; also popular are *Bidens pilosa* Linnaeus and *Verbena* spp. All of these are either grown in the insectaries or provided daily as freshly cut flowers.

Small cubic cages measuring about three feet on each side served to isolate newly emerged imagos in preparation for experimental work. Individuals could be thus kept for about two days without harm to their subsequent vitality, providing that the cage stood in the shade, was partly covered with green branches and was freely sprinkled with water during the heat of the day. *Lantana* was supplied on a shelf six inches below the roof, since butterflies kept in

such small spaces do not readily feed from bouquets on the floor. Although mating was once secured in a small cage, normal courtship was absent, and no female was ever induced to oviposit under such conditions.

A description of the growth stages of this species, as well as an account of the rearing technique, is in preparation. Here it is appropriate to mention the general precautions necessary in order to produce the vigorous adults which are essential for behavior study: the larvae must be reared in individual, closely covered containers; provided amply at least once a day with freshly picked, or briefly refrigerated, leaves of appropriate sizes from the vine *Passiflora tuberosa* Jacquin; furnished with suitably high amounts of light and humidity; and kept clean. The chrysalids also must be kept in a humid environment. In short, this is not a species that can be healthfully reared *en masse* in a shoebox.

The experimental techniques employed in the study of vision and of social behavior will be described under the appropriate sections (below and pp. 183-190).

IV. Vision: Spectral Range and Color Perception

In this section will be briefly considered certain characteristics of the visual sense of *Heliconius erato,* with brief mention of other butterflies.

A few experiments were made, as described below, only to establish with certainty that color perception and sensitivity from the near ultraviolet at least to the orange exist in this species and play a role in both innate and acquired feeding responses. Detailed studies of color vision and feeding behavior are reserved for the future.

A. Limits of the Spectrum. From time to time various species of butterflies, including *H. erato,* were tested for responses to light of various spectral characteristics. In each experiment a wooden box was used, measuring either 3′ × 3′ × 2′ or 4′ × 3′ × 3′. Each box was completely light-tight, all cracks and corners being reinforced with black photographic tape, except for the following apertures: One 5″ × 5″ opening in a corner of the top, for covering with a filter which, in turn, was sealed in place with tape; a light-tight door, near the bottom, for insertion of butterflies; a suitably-sized hole for insertion of a flashlight, which was fastened tightly with the switch extending outside; and finally an eye-hole. The latter measured about 1¾ inches in diameter and was fitted with a short length of flexible black tubing; when not in use the exterior end of the tube was covered with a black paper cornucopia. Before every test the box was checked for light-seepage by an independent observer.

After the introduction of one to three butterflies the box was left undisturbed in the open air for a maximum of five minutes. When the ultraviolet filter was used on dull days, the natural daylight was supplemented by placing a portable ultraviolet lamp close above the filter. After a few minutes, the positions of the insects were observed by means of the eye-hole and, in the case of the ultraviolet filter, by use of the inserted flashlight. The filter was then covered with a piece of wood and, when the observer's hand was inserted through the butterfly entry door, the butterflies were disturbed and forced to seek new positions away from the filter. The same procedure was then repeated twice more with all individuals tested.

In the tests involving *H. erato,* 11 out of 12 specimens individually introduced under each of the filters (not necessarily on the same day) batted against both the Corning Ultraviolet #5860-7-37 and Corning Red #2408-2-60. These filters pass, respectively, no light of wavelengths longer than 390 mμ or shorter than 610 mμ. Most of the responses to the ultraviolet occurred within 30 seconds. Strong positive responses were also obtained to filters transmitting freely in intermediate spectral regions. The following other species were tested with similarly unequivocal results: Heliconiidae: *Heliconius sara* (3 individuals), *H. ricini* (2), *Agraulis vanillae* (3). Danaidae: *Danaus plexippus* (4). Ithomiidae: *Tithorea mopsa* (1). Papilionidae: *Papilio neophilus* (3), *P. anchises* (2), *P. anchisiades* (3). Pieridae: *Eurema albula* (2), *Phoebis sennae* (2), *Anteos maerula* (1).

Although it was expected that butterflies would prove to be sensitive to the ultraviolet, in common with the numerous insects of other orders which have to date been tested, no reports on such responses in Rhopalocera appear hitherto to have been published.

The tests established conclusively that for *H. erato* the visible spectrum extends from at least the near ultraviolet at least up to 610 mμ, which marks the extreme lower transmission limit of the red filter employed. The experiments yield no data at all, of course, concerning the relative brightness for the insect of the various spectral regions, nor do they indicate whether any of these regions are distinguished qualitatively, that is, as different colors.

B. Color Perception. Since existence of a color sense in the heliconiids has never previously been tested, basic experiments were undertaken to determine whether the capacity is pres-

ent in this family. The same general system was used as that employed by von Frisch and others with bees (von Frisch, 1948, 1950 and ref.), and by Ilse with butterflies (1928). In all of these, colored samples, in our case paper flowers, were offered among a large number of gray samples ranging in brightness from "white" (positively ultraviolet), to "black." No training was undertaken in the present series, since under certain conditions the species came spontaneously to the colors, and since it was only desired to determine whether or not they could distinguish color from any shade of gray, rather than one color from another.

TEXT-FIG. 1. Pattern used in making three dimensional paper flower. The fringed strip is rolled up and fastened at the base with scotch tape and a paper clip. Natural size.

In the present tests, advantage was taken of the tendency of *H. erato* to come to small colored objects of certain forms—i.e., of flower-like shape. As has been shown in bees (von Frisch, 1948, 1950 and ref.) and butterflies (Ilse, 1932.2), the most successful were small circles with fairly numerous converging petal-like divisions. Our most successful models were more complex than those previously described, and strongly three-dimensional. Flat ones, although they evoked a few responses, were far less popular.

Series of these model flowers were fashioned from Stoelting Psychological Test papers in 17 colors, and from papers painted in a variety of opaque water colors and with Floquil paints. All of these were analyzed spectrophotometrically in the visible and near ultraviolet through the courtesy of the Research Laboratories of the Interchemical Corporation.

A series of 20 gray steps was also made, by hand, from highly ultraviolet reflectant blotting paper dipped in a measured succession of progressively higher dilutions of India ink with distilled water. This laborious procedure was necessary because a commercially manufactured gray-step series with the necessary characteristics could not be located. The only one found in the United States (the Munsell series) with a sufficiently matte surface proved upon spectrophotometric analysis to have a very low reflectance in the ultraviolet. In fact some results obtained during one season were misleading until this characteristic was ascertained, since the butterflies came rather freely to some of the lighter grays; apparently for them, as for bees, negatively ultraviolet whites are colored. This never occurred with the India ink series, the only responses to uncolored (for humans) flowers being to a model painted with Chinese white (zinc oxide); as is well known, this substance has minimal reflectance in the ultraviolet, and so, by subtraction, apparently appears colored to the butterfly.

In order to control the possibility that the odor of the India ink was a deterrent to the butterflies or that, conversely, some of the paints or papers had an odor attractive to them, the same ink, in various dilutions, was applied in alternate rows of "petals" on single colored flowers, while bits from the most popular colored flowers were placed invisibly but open to the air beneath as well as within some of the gray models. The butterflies' responses were unaffected. The preference of the butterflies for three-dimensional models made impractical von Frisch's use of a glass plate over flat models.

The technique of the tests was as follows: they were run in two parts. First, a group of experienced butterflies, well settled in an insectary, was deprived of food for a few hours. The best time for tests was between nine and eleven o'clock on a sunny morning; therefore food was usually removed the preceding afternoon. The artificial flowers were displayed in checkerboard fashion on an inverted square tray made of wire netting with a one-quarter- or one-half-inch mesh. The flower models were attached to the meshes by paper clips. They were displayed both with and without basal circles of black paper for contrast, without noticeable difference in the results. Sufficient tests of this aspect, however, have not yet been made which would enable the statement of reliable conclusions.

Freshly picked *Lantana* leaves were used to cover the netting at the base of the models, since experienced butterflies made extremely few responses to models without the added stimulus of odor. Amyl acetate was also used with some success, being sprayed on a pan of damp sand or earth in which the models were set. In half the tests no reward was provided; in the other half, all the models—both colored and gray—were fastened to half-dram vials filled with a 10% solution of white sugar.

TABLE 1. SPECTRAL REFLECTANCE OF PAPERS, PAINTS AND TEXTILES USED IN *Heliconius* EXPERIMENTS.

Explanation: From spectrophotometric curves furnished by the research laboratories of the Interchemical Corporation, New York, N. Y. Sources and general type of the materials used were as follows:

Papers: a. Stoelting Psychological Test series (a smooth-finish matte surface).
b. A series of school construction papers (a rough-finish matte surface).

Paints: a. Floquil "Flopaque" (a quick-drying opaque lacquer).
b. Richart opaque water colors.
c. "Stroblite" fluorescent paint. (Translucent). (Bright pink).

Textiles: a. Light-weight canvas.
b. Heavy, opaque, felt-like cotton.

The papers were used for experimental flowers and markings on butterfly models; opaque lacquer used especially for painting living butterflies, but also for paper flowers; opaque water-colors principally for paper flowers; canvas and felt served as foundations for butterfly models.

Also used were (1) white paper and paint with high ultraviolet reflectance which approached that of wavelengths in the visible; (2) zinc oxide paint with minimal ultraviolet reflectance; and (3) a series of 18 neutral grays, with positive ultraviolet reflectance (see text, p. 171).

			REFLECTANCE (% OF MAGNESIUM OXIDE)																	
			Ultraviolet			Violet		Blue		Blue-green		Green		Yellow	Orange		Red			
			Wave-length (mμ)																	
Sample	Color	Material	340	360	380	400	420	440	460	480	500	520	540	560	580	600	620	640	660	680	700
1	Violet	Paper	18	20	25	26	27	30	28	20	13	7	8	4	5	8	9	11	13	18	25
2	Violet	Paint	33	31	38	43	46	45	36	23	14	9	8	8	9	9	12	19	35	57	74
3	Violet-blue	Paper	9	10	14	19	22	23	22	18	14	11	9	8	7	7	10	17	23	27	33
4	Blue	Paper	9	10	16	35	46	52	51	39	24	14	10	7	6	6	6	7	8	12	18
5	Blue	Paint	8	8	12	23	28	29	28	25	21	17	15	12	11	10	9	8	7	7	7
6	Blue	Paint	52	55	59	56	61	66	64	52	37	24	16	12	11	10	11	12	15	20	28
7	Blue	Cloth	21	24	30	31	35	36	36	33	29	25	21	18	16	15	15	16	17	19	21
8	Blue	Paper	17	20	24	24	25	38	45	46	45	40	30	20	12	9	7	6	8	12	23
9	Blue-green	Paper	10	10	14	23	26	29	35	46	54	53	47	40	32	27	24	22	24	28	32
10	Blue-green	Paint	7	8	14	22	24	25	28	32	33	31	28	25	21	18	16	14	13	12	12
11	Blue-green	Cloth	18	17	15	14	14	16	22	30	34	32	27	22	17	13	12	13	14	16	18

12	Green	Paint	6	6	6	5	5	6	7	11	17	18	15	11	9	8	7	6	6	6	6
13	Green	Paint	14	14	13	11	10	18	18	27	38	38	31	23	17	13	11	9	9	12	18
14	Green	Cloth	8	8	8	7	7	8	11	17	22	23	20	16	12	10	9	9	9	10	11
15	Yellow-green	Paper	14	22	24	20	20	21	24	30	45	56	58	45	40	38	37	38	41	44	48
16	Green-yellow	Paper	13	14	14	13	12	13	15	19	35	61	67	62	55	51	48	46	50	59	69
17	Yellow	Paper	7	7	9	8	9	10	14	25	41	59	67	70	71	71	70	70	72	75	77
18	Yellow	Cloth	9	9	8	9	8	9	11	15	18	34	41	46	50	53	55	58	60	62	64
19	Yellow	Paper	9	9	12	16	17	17	19	26	36	44	51	57	62	66	69	72	74	77	79
20	Yellow	Paint	5	6	6	6	6	6	6	7	9	25	52	65	71	73	74	75	76	76	77
21	Yellow	Paint	8	8	8	8	8	8	9	11	22	50	75	83	84	85	85	86	88	90	91
22	Yellow-orange	Paper	7	7	7	6	7	7	7	7	9	25	54	68	72	74	75	76	77	78	79
23	Orange	Paper	6	5	5	6	5	5	5	6	6	9	10	15	33	58	70	73	75	77	78
24	Orange	Paper	7	9	10	10	10	10	8	7	7	7	9	19	39	55	62	66	69	72	74
25	Orange	Paint	7	7	7	6	6	7	7	7	8	11	17	29	45	61	71	76	78	79	80
26	Orange	Paint	13	12	12	11	10	10	10	10	11	12	14	23	51	75	82	84	85	88	90
27	Orange	Cloth	8	12	10	7	6	6	5	6	6	8	10	22	41	55	60	62	63	65	68
28	Orange-red	Paper	6	6	6	5	5	5	5	6	6	7	7	9	16	39	65	75	78	79	80
29	Red	Paper	6	6	5	4	4	4	4	4	4	5	5	6	8	19	45	65	72	75	78
30	Red	Paper	7	7	10	10	9	8	7	6	5	5	6	7	11	17	26	38	50	59	65
31	Red	Paint	4	4	4	4	4	4	4	4	4	4	5	5	8	13	33	57	67	72	74
32	Red	Paint	15	20	16	16	16	16	13	11	10	9	9	8	18	38	70	82	86	88	90
33	Red	Cloth	3	4	4	3	3	3	3	3	2	2	2	3	4	10	25	40	50	57	62
34	Violet-red	Paper	14	16	22	21	21	23	22	16	11	8	7	6	10	22	45	56	61	64	67
35	Pink	Paper	14	17	18	17	16	16	14	15	11	10	9	11	19	40	64	70	73	75	76
36	Pink, fluorescent	Translucent Paint on White Paper	10	10	15	21	25	31	34	27	22	20	19	20	20	40	63	68	72	75	77

In preliminary tests five models were displayed, representing general spectral regions: blue, blue-green, yellow and orange-red, all except some blues having minimum reflectance in the ultraviolet. As always they were exhibited with the complete series of gray models, plus one of Chinese white. In subsequent tests four models, of roughly similar hues but of varying brightnesses and texture, were displayed among the grays, such as four yellows, or four orange-reds and reds. Generally each test was limited to 15 minutes. Hungry butterfles on bright days would return and probe a paper flower—whether or not sugar water was attached—a number of times; well-fed individuals in cloudy or oppressive weather would give very few or no responses, even though they were repeatedly flushed from resting positions. Responses were divided into three parts (Table 2), "dips," in which the butterfly changed course and fluttered down within three inches of a particular model, alightings and probes; in the latter actual and often vigorous attempts were made to feed, with the proboscis uncoiled; when no sugar water was furnished the attempts were sometimes much prolonged.

Second, inexperienced butterflies, which had never previously fed on or seen any flowers, or even any other butterflies, having been kept in isolation cages (p. 187), were introduced singly into an insectary. Individuals, especially males, allowed to go about fifty-four hours without food upon emergence, sometimes came spontaneously straight over to the experimental tray and probed repeatedly at several differently colored models in succession, a variety of hues being displayed among the grays. These responses were without the added attraction of either *Lantana* leaves or amyl acetate, and no sugar water was provided. In fact, no chemical stimulus was ever employed with the inexperienced individuals; therefore their positive responses were all to visual characteristics alone. However, younger butterflies had to be tested by fastening the test flowers in succession on the end of a stick; the model was then brought, sometimes several times over, slowly up to the head of the resting butterfly. The order of color presentation was varied in different individuals, and with the same individual in different test sessions, without allowing either real food or other insects in the insectary until after the conclusions of the tests.

The results of both these groups of preliminary tests to determine the presence of color vision in *H. erato* were conclusive:

1. Neither experienced nor inexperienced butterflies ever dipped toward, settled on or probed an uncolored paper flower, except for the model painted with negatively ultraviolet Chinese white (zinc oxide).

2. Positive probing responses were obtained for literally all regions of the spectrum, including some with relatively high ultraviolet reflectance, except blue-greens and greens, and by inexperienced as well as experienced butterflies, without the addition of odor. Feeding response to color is therefore innate.

3. The largest numbers of responses were received, in this order, by yellow, orange-yellow, orange and Chinese white. Blue, whether positively or negatively ultraviolet, was next to blue-green and green least popular of the colors. No actual proportions of responses to the different models can be given; the variations of presentation were not made with sufficient care to ensure a random, or other system of rotation, and a given butterfly, or group of butterflies, invariably ceased to respond before an entire series could be run. Table 2 gives an adequate sample of typical series of responses.

4. The preferences held among the inexperienced as well as the experienced and show that the attraction for "yellow" in food is innate and not only a learned association with such favorite food-flowers as *Lantana, Bidens,* etc. It is, of course, by the terms of the experiments, not yet known whether the unpopularity of the blue and green regions is due merely to a low retinal sensitivity. It is interesting to recall that in Ilse's experiments, vanessids—which of her groups are nearest the heliconiids—found the blue and yellow regions of the spectrum most attractive. It will be noted that blue is not a favorite with the heliconiids. The papilionids and pierids on the other hand came most freely to red. Casual but repeated observations in the field, garden and insectaries in Trinidad support this red preference in the tropical papilionids and pierids.

V. Behavior of Imagos: General Account

A. Introduction. The following account of the adult behavior of *Heliconius erato* is based principally on the activities of specimens observed in the Trinidad insectaries. However, all the general observations, including food flower preferences, time of day and meteorological conditions governing roost-leaving, feeding, courting, egg-laying and roosting, have been checked a number of times in the field, by other staff members as well as by the author. These checks were found to agree with the more detailed observations made possible by the insectaries.

B. Habitat and Flight Habits. *Heliconius erato* flies typically along the edges of seasonal, lower montane and swamp forests, and of well-

TABLE 2. FEEDING RESPONSES OF EXPERIENCED *H. erato hydara* TO 4 COLORED ARTIFICIAL FLOWERS AMONG A SERIES OF 18 GRADUATED GRAYS AND ZINC OXIDE WHITE.

The data below are typical of the results obtained in about twenty similar tests. See text, p. 170 ff. and Text-fig. 1. Numbers under colors refer to samples analyzed spectrophotometrically in Table 1, p. 172.

Test conditions: Five hungry individuals were used in each 15-minute test. No individual had been tested within the preceding five days and all were habituated to insectary conditions. Tests were conducted only on calm, sunny mornings between 9:30 and 11:30 A.M. No food was furnished with the flower models, although *Lantana* leaves were placed evenly around their bases. The models were arranged in the usual checkerboard (Roman square).

In no test was there any response whatever either to models of the gray series, including positively ultraviolet white, or to *Lantana* leaves.

Test a

Responses to Violet, Blue and Zinc Oxide White

	Violet (No. 1)	Violet (No. 2)	Blue (No. 6)	Blue (No. 13)	Zinc Oxide White
Dips	–	–	–	–	–
Alightings	–	–	–	–	2
Proboscis probes	–	1	–	1	2
Total	–	1	–	1	4

Test b

Responses to Blue, Blue-green and Zinc Oxide White

	Blue (No. 5)	Blue (No. 9)	Blue-green (No. 10)	Blue-green (No. 12)	Zinc Oxide White
Dips	1	–	–	–	3
Alightings	–	1	–	–	2
Proboscis probes	–	–	–	–	4
Total	1	1	–	–	9

Test c

Responses to Yellow and Zinc Oxide White

	Yellow (No. 19)	Yellow (No. 20)	Yellow (No. 21)	Yellow-orange (No. 22)	Zinc Oxide White
Dips	2	8	1	13	2
Alightings	–	–	1	2	1
Proboscis probes	16	2	21	6	2
Total	18	10	23	21	5

Test d

Responses to Orange, Red and Zinc Oxide White

	Yellow-orange (No. 22)	Orange (No. 23)	Orange-red (No. 28)	Red (No. 29)	Zinc Oxide White
Dips	7	4	2	1	1
Alightings	1	2	–	–	–
Proboscis probes	9	5	–	–	–
Total	17	11	2	1	1

established second growth. These characteristic localities occur along roads, open trails, streams and old, small clearings; the butterfly is also common in overgrown citrus and cocoa cultivations. It does not fly in the depths of thick forests, since partial sunlight is an essential. In the Arima Valley it has not been seen above 1,200 feet, although it was fairly common at

Rancho Grande, Venezuela, at 3,600 feet. It is a low flier, usually fluttering between about three and seven feet off the ground, and rarely is seen as high as 20 feet. Females, when about to lay, skim along a few inches to two feet above the ground, since the local food plant is usually low-growing.

In the insectaries a combination of ample shade and sunlight is required plus a high humidity and an abundance and variety of growing herbs and shrubs. Given these conditions *H. erato* thrives in captivity better than any of the remaining fifty-odd species so far tested, although all the heliconines do well.

C. Relation of Behavior to Meteorological Conditions. Temperature, light and humidity are all important in the behavior of these completely diurnal butterflies. Temperature is the critical factor which governs their first morning activity, while light intensity controls roosting in the late afternoon. At Simla they are always inactive, regardless of the light intensity, at temperatures lower than 67° F. (19.4° C.); the usual minimum for activity however is 70° F. (21.1° C.). This temperature has never, between December and June, which includes the coolest season, been reached later than 8 A.M. The usual time of first morning activity changes with the seasons. In January it usually occurs between 7 and 8, in April between 6:30 and 7:30, and in May and June the first butterflies frequently leave the roost at 6:45. In hot weather, when the early morning temperature does not fall below 70° F. (21.1° C.), light is the crucial factor. Ground light must usually be around 3.2 by Weston exposure meter, south light 25-50, and overhead sky light (through the insectary roof), 25-100; however ground light may be as little as 1.6 and sometimes registers more than 6.5 before the first flights are made. A single butterfly once flew, at 5:45 A.M. in late April at a temperature of 73° F. (22.3° C.) when the ground light registered only .4, the south light 3.2 and the sky between 3.2 and 6.5. For comparison, noon conditions on a bright sunny day with a typical rainforest combination of some clear sky and some clouds show the following readings inside the insectary: ground 25-50, south 200, overhead sky 800. Full morning activity by all healthy individuals is reached when the temperature is at least 73° F. (22.3° C.) and the ground at least 13 Weston in some areas, south light 50 and overhead sky more than 50. Activity is always higher on sunny days.

The butterflies become inactive in rainy, dull or windy weather. They also often become inactive up to an hour before a heavy squall, even while the weather remains calm and bright. An attempt to link this with barometric pressure failed. On hot, dry afternoons the butterflies tend to fly high and erratically when the humidity drops below fifty per cent., batting against the roof and becoming exhausted. Sprinkling the insectary at once corrects this condition.

In bad weather, roosting—that is, hanging upside down from a twig or tendril, with the wings closed—may begin as early as 2:30 in the afternoon. On bright or moderately cloudy days, however, the first butterflies may start gathering in the roosting place around 4:45 P.M. in the average month of April, while others are still feeding. The majority hang up around 6:00 P.M. and all are in their final positions at 6:15. The time varies only about twenty minutes one way or the other from these hours on the longer and shorter days around the solstices. The temperature records during the going-to-roost period have ranged from 80° F. (26.7° C.) down to 73° F. (22.8° C.), and, as was said above, did not apparently affect the roosting behavior, which was primarily controlled by the decreasing light. When the ground-vegetation light recorded between 6.5 and 13 Weston, with the south over 25 and the sky overhead more than 50, nearly all the butterflies continued to feed, and courting sometimes took place. At intensities less than the above, the butterflies gradually gathered to roost. In one instance, an individual fed accurately and subsequently found the roost at the extremely low intensities of ground-vegetation .4, south 3.2-6.5 and sky 13. Feeding with a ground reflectance of 1.6, south 6.5-13 and sky 13-25 was not uncommon. It will be seen that these intensities are slightly lower than those at which the first morning activity usually occurs, when the tempertuare is nearly always lower than the low of 73° F. (22.8° C.) recorded for going to roost.

The conditions controlling first morning activity and roosting are summarized in Table 3. Although observations on other species are incomplete, it may be said here that forest-living ithomiids are active at both lower light intensities and lower temperatures, while *Heliconius sara* and *H. ricini*, which fly in slightly more open areas than *H. erato*, require either more light or higher temperature or both for activity. These characteristics lead, in the insectaries, to the ithomiids flying both earlier in the morning and later in the afternoon than *erato*, while *sara* and *ricini* both usually leave the roost later and hang up on it earlier than does *erato*.

D. Feeding. *H. erato* is altogether a flower feeder, never coming to fruit. From observations on feeding preferences in the insectaries, garden and in the field, Table 4 was compiled. *Lantana* has been observed to be strongly attractive in

TABLE 3. RELATIONSHIP OF DIURNAL ACTIVITY TO TEMPERATURE AND LIGHT IN *Heliconius erato hydara*

Based on observations made in Trinidad, B.W.I., between December and June, altitude 800 feet. All light readings made under wire netting roof of insectary, with Weston photometer pointing directly overhead. For fuller data, see text.

	MORNING (First activity: 5:45-8:00 AM) Light always more than 25 Weston			AFTERNOON (Last activity: 4:30-6:00 PM) Temperature always more than 73°F.		
Activity	Temp. < 67° F.	Temp. 67°-72° F.	Temp. > 73° F.	Light > 100 W.	Light 25-100 W.	Light < 13
Full	–	–	X	X	–	–
Slight	–	X	–	–	X	–
None	X	–	–	–	–	X

the field in both Venezuela and Surinam, as well as in Trinidad, and is far and away the favorite in the insectaries, being used as the staple diet.

Feeding takes place all during the day, but is especially active on bright mornings between 9 and 11 A.M. When left undisturbed in the insectaries, neither sex seeks food until 20 to 24 hours after emergence; however, when presented with food some individuals will uncoil the proboscis and feed about six hours after emergence.

E. SOCIAL BEHAVIOR. Social behavior may be divided into three categories, namely, courtship, social chasing and roosting. The following paragraphs are descriptive statements; the activities will be analyzed in subsequent sections (p. 183 ff.).

1. *Courtship.* In the normal sequence, two alternative beginnings are possible. First, a male gives chase to a passing receptive female and pursues her closely. She usually settles on a fairly broad green leaf within a few moments, sometimes apparently urged to alight by the male's flying slightly above her and by actually brushing her wings with his on the downbeats, thus forcing her downward. Alternatively, a male approaches a female at rest on a leaf; her wings may be open or closed. Females which have already been stimulated by unconsummated courtships often will remain on the same leaf for hours, responding promptly to subsequent males.

At the male's approach, the female usually opens and closes the wings slowly several times. If she is receptive and begins to court, however, both pairs are held closed over the back. The male begins (Plate I, Figs. 1-3) by flying in short, repeated spurts close behind her, hovering in place, his forewings sometimes almost touching her hindwings and creating a current of air against them. Occasionally on a downbeat the male wings actually cover those of the female (Plate I, Fig. 4). During this stage, as well as in any preliminary chasing of the female, his fore- and hindwings are not separated to expose the silvery friction surfaces which apparently distribute the products of the scent scales (see p. 181). The male harpes are not in evidence, the tip of the abdomen remaining tightly closed, and no odor is detectable in this sex at this stage to the human observer.

The response of a receptive female to this buffeting is shown in two ways: first the abdomen is elevated and a gland, bright chrome yellow in hue, is extruded dorsally at the junction between the penultimate and distal segments. Two bulbous excrescenses project from it, one on each side of the dorsal midline, and are arhythmically inflated and partially deflated with waxing and waning stimulation. Below and behind them is the pair of tiny "stink clubs," described, along with the gland, in other heliconines by Müller (1877, 1912), and in this species by Eltringham (1925). These clubs are extremely difficult to see, even with a hand-lens, in the actively courting butterfly, and, indeed, are not exserted except during maximum excitement; however they are unmistakably in evidence in some frames of our motion pictures of courting females. The clubs show up as yellowish brown, in contrast to the chrome yellow of the gland proper.

Usually no odor is detectable to the human observer during this response of the female; rarely a musky odor is evident at very close range in individuals less than about two days old. Apparently this is the same odor which is detected when females are allowed to emerge from the chrysalid in a closed box; young males have, to human nostrils, a similar odor. Only in females which have been already mated and are being persistently courted on the following day, or, rarely, in which advanced courtship is disturbed so that the insects fly off with the female gland exserted, is a witch-hazel-like odor dis-

cernible during courting. This odor, well-known in the species, has no apparent effect on the courting male. (cf. "Defense," p. 180). Ford (1945, p. 96) has remarked that, unlike the products of male scent scales, the chemical attractants of female butterflies have not been reported as perceptible to human observers. Except for the circumstances just noted, this is true also of *H. erato*.

As the tip of the abdomen is elevated and the gland exserted, the anterior margins of the forewings, as they are held closed above the back, are pressed closely together. The more posterior portions of the forewings are allowed to bell outward. Simultaneously the hindwings are partly opened and rapidly vibrated for a second or two with brief intervals between vibrations.

The next stage of courtship, Stage II, may be omitted or strongly curtailed by excited butterflies when the female is highly receptive. Nevertheless, it is usually both well-marked and characteristic. The male shifts position from the rear forward, so that his flapping continues above her and, especially, immediately in front of her. He continues to face in the same direction as the female, or sideways, and he may or may not at times touch her forewings with the tips of his hindwings. His fore- and hindwings are now separated, sometimes widely, exposing the silvery friction surfaces (Plate I, Fig. 5;

TABLE 4. FEEDING PREFERENCES OF *Heliconius erato hydara* IN TRINIDAD.

(Representative examples of both wild and garden species are included. Colors are roughly indicated of entire inflorescence, including bracts or rays, to give an idea of their variety. None of the whites and very few of these colors reflect more than minute amounts of ultraviolet. The important roles played by form and odor in the relative attractiveness of the different species are not considered here.)

	FREQUENTLY VISITED	
Loganiaceae.	*Buddleia variabilis* Faranchet.	Buddleia. Purple.
Verbenaceae.	*Lantana camara* L.	Lantana. Yellow & orange.
	Verbena spp.	Vervain. Purple; white.
Rubiaceae.	*Cephaelis tomentosa* Willd.	Wild Ipecancuanha. Yellow & red.
	Warszewiczia coccinea. (Wahl) Kl.	Wild Poinsettia. Yellow & red.
	Hamelia erecta Jacq.	Wildclove. Orange.
Boraginaceae.	*Cordia cyclindrostachya.* R. & S.	Black sage. White.
Compositae.	*Bidens pilosa* L.	Spanish Needles. Yellow & white.
	Senecio confusus Britten.	Gem of the Rio Grande. Orange.
	OCCASIONALLY VISITED	
Zingiberaceae.	*Costus spiralis* Rosc.	Scarlet Cane Reed. Yellow & red.
Orchidaceae.	*Epidendron fragrans* Swartz.	Purple Streak Orchid. White with purple streaks.
	Oncidium luridum Lindl.	Brown Bee orchid. Yellow & brown.
Asclepiadaceae.	*Asclepias curassavica* L.	Milkweed. Yellow & orange.
Solenaceae.	*Browallia americana* L.	False violet. Yellow & violet.
Gesneraceae.	*Tussacia pulchella* Rchb.	Harlequin Flower. Yellow, orange & purple.
Cucurbitaceae.	*Momordica charantia* L.	Carilla. Yellow.
	RARELY VISITED	
Musaceae.	*Heliconia hirsuta* L.	Paradise flower. Yellow & orange.
	Heliconia humilis Jacq.	Swamp balisier. Yellow & orange.
Bignoniaceae.	*Tabebuia serratifolia* (Vahl).	Poui. Yellow.
Rubiaceae.	*Ixora coccinea* L.	Ixora. Red.
	APPARENTLY NEVER VISITED	
Papilionatae.	*Erythrina micropteryx* Poepp.	Mountain immortelle. Orange-red.
Convolvulaceae.	*Convolvulus* spp.	Morning glory. Blue; yellow; white.
Bignoniaceae.	*Tabebuia pentaphylla* Hemsl.	Poui. Pink.
Malvaceae.	*Hibiscus* spp.	Red; pink; orange; yellow; white.
Plumbaginaceae.	*Plumago capensis* L.	Plumbago. Blue.
Campanulaceae.	*Centropogon surinamensis* (L.)	Presl. Crepe Coq. Red.
Acanthaceae.	*Pachystachys coccinea* Ns.	Black Stick. Red.

Plate II, Fig. 11). Both fore- and hindwings are in rapid motion (Plate II, Fig. 12), unlike normal flight, where the hindwings are almost motionless. A flowery fragrance can rarely be detected by the observer at this stage, probably emanating from the now-exposed friction surfaces. The tip of the male abdomen is still tightly closed. When both sexes are subsequently examined, both fore- and hindwings, especially near the bases and along the anterior margins of the hindwings, as well as the thorax, are found to be similarly fragrant. The female fragrance develops even in uncourted individuals, although to a lesser extent, and hence must be a product on her own, rather than merely sprayed on by the male in Stage II (see p. 182).

During Stage II the female continues to vibrate the hindwings while the anterior margins of her forewings remain closely apposed. When Stage II is prolonged, however, in its extreme form where the male fans her from a position in front of her, rather than directly above, she withdraws the abdominal scent gland and partly lowers the abdomen. Her antennae are occasionally lowered during Stage II, again principally when the male is courting from in front of her.

The final sequence, Stage III, immediately precedes copulation. It begins with the male's alighting and moving backward beside the female, on either side, but more frequently on the right. He still faces in the same direction as she, and he usually comes to rest with his eyes near the level of her thorax. He continues flapping his wings with the friction surfaces exposed and she once more elevates the abdomen and extrudes the scent gland. Fragrance is most likely to be detected at this stage, but its source cannot be determined because of the insects' activity. If Stage II has been omitted, the male simply alights beside her, his friction surfaces now being exposed for the first time. In any case he extrudes the harpes, twists his abdomen sideways, and seeks to curve it forward between the posterior margins of the female's now folded hindwings until the harpes can engage the female's abdomen. In fully receptive females the abdomen is by now lowered, the scent gland being simultaneously withdrawn. The harpes grasp the abdomen's lower tip, gripping it by the latero-ventral portion of the penultimate segment.

During copulation, which usually continues more than an hour and rarely overnight, both pairs of wings in both sexes are normally closed and held erect over the body in typical daytime rest position. The male swings promptly around so as to face in the opposite direction from the female. When disturbed a short flight is made to another resting place, the male carrying the inert female. No odor is apparent during copulation except for an occasional faint fragrance when, if slightly disturbed, the male slowly waves his wings.

Persistently courting males which are not accepted by females, or old males which have made repeated unsuccessful attempts to engage the female's abdomen, or males which have been courting females of another species (namely, *Heliconius melpomene, H. ricini, H. isabella,* and *Dryas julia*) sometimes come to rest in front of the female, facing her, which is a position atypical for this species. Alternatively, they may sit quietly beside her, their heads on a level with the female's head, or, more often, her thorax. From either position the male sometimes palpates the female's head, antennae or thorax with his antennae, and, rarely, with his uncoiled proboscis (Plate III, Figs. 13, 14). Another type of atypical behavior often occurs among the old males, which court facing the female, and flying both in front of, above and behind her, in irregular alternation; the males' friction surfaces remain exposed regardless of their position. None of the atypical behavior described in this paragraph occurs in the course of a normal courtship. It appears exceedingly likely that displacement behavior is involved, particularly when the proboscis is inappropriately extruded.

Courting may take place at any time during the day but is especially common during the late morning and after 2:30 P.M. Males almost never court females engaged in feeding at bunches of flowers, although they are not similarly restricted if the female is on an isolated blossom. There is no courting on the roost (see below).

Sometimes a receptive female which has not been courted will flutter the hindwings at the close passing of almost any species of butterfly; if she is more than two days old and still unmated, she may freely chase passing *H. erato* of any age; younger females, however, fly and feed very little.

Males and females both can mate at least twice, the males from the second to at least the thirty-first day after emergence. Some individuals court persistently but unsuccessfully for another month. Females have been seen mating from 45 minutes after emergence through the seventh day. The hindwing flutter and extrusion of the scent gland in response to courting takes place through the eighth day. However, the anterior forewing margins are held less and less tightly together following the third day, and the abdominal gland is extruded less and less completely.

2. *Social Chasing.* Chasing-and-circling, which seems not to be directly of a sexual nature, is a frequent activity of healthy male butterflies throughout their lives and of females which have completed egg-laying. The males and old females chase both one another and non-receptive females indiscriminately in short flights, sometimes with mutual circlings. Young males and newcomers to the insectary are especially subject to chasing. There is no apparent attempt to force a chased butterfly to alight, as in preliminary courtship, and the roles of chaser and chased are frequently reversed several times during a flight. The abdominal gland of the female is not exserted, nor are the male harpes, the friction surfaces are not exposed and there is no detectable odor of any kind. The flights are never the result of two or more males courting a single female, although this multiple courting often takes place. The chasings and circlings invariably end quite simply with the two or three butterflies involved going their separate ways.

Sometimes a butterfly will approach a resting individual, which is not a receptive female, from the rear, as in the first stage of courtship. The resting butterfly then opens and closes the wings slowly a few times, whether they have been held open or shut, whereupon the approaching individual flies away.

Except for the apparently non-courtship relationships described above, no approach has been found to inter-male threat display or fighting, nor is there evidence in the insectaries of territoriality or of a dominance order. Males and old females are far more active in both chasing and feeding than females which have not completed egg-laying.

3. *Roosting.* During the late afternoon *H. erato* follows the family habit of gathering in groups for the night. In the insectaries as in the field the butterflies often crowd the same dead twigs or vine tendrils, night after night, hanging upsidedown, the wings closed. Species of the same genus may roost with them. In the insectaries males and old females tend more to occupy crowded roosts together, while younger females, except when at their most receptive (the second through the fourth days), show less gregariousness. However, these receptive females usually roost on the more crowded perches, or sometimes next to a somewhat isolated male. No courtship takes place on or near the roosts, although there is always a great deal of activity, buffetting and pushing and jockeying for position, as the butterflies gather.

F. Defense. No organized work on the distastefulness of *H. erato* has yet been undertaken in the course of the present study. However, many casual tests have been conducted in our laboratories, both at Rancho Grande, Venezuela, and in Trinidad. Almost all of these confirm the heliconiid reputation of distastefulness to their natural predators. Butterflies of both sexes and all ages have been refused by various individuals of the lizard, *Polychrus marmoratus.* Some were seized by the wings or body and dropped, while others were disregarded altogether. In some butterflies the scarlet band was cut off by the observer before the insect was offered to the lizard. Individuals were also refused by the frog, *Hyla maxima.* Usually, but not always, they were refused, or examined and dropped, by captive capuchin monkeys. The butterflies were always either dropped or disregarded by the mantids *Stagmatoptera septentrionalis, Stagmomantis carolina* and *Oxyopsis rubicunda.* All of these laboratory test animals, from lizards to mantids, freely ate Lepidoptera of unprotected families and comparable size following the refusal of *Heliconius erato.* Ponerine ants, however, attacked and ate ailing *Heliconius* in the insectaries, and various non-ponerines scavenged them freely. Also, several species of epeirid spiders were major insectary enemies.

In *H. erato* three or four different odors may be distinguished by the human observer. All of them probably play roles in the insect's sexual or social patterns or in both. At least two of them and possibly all appear to be concerned also in the butterfly's defense. These odors and their sources will now be considered in turn.

1. *Abdominal Glands.* It has been generally held in the literature (see ref., p. 169) that the glands of the penultimate abdominal segment in the female, and in each harpe of the male are aposematic in function, discouraging attack by emitting an unpleasant odor. The scent has been variously compared with that of carbylamine, phenyl carbylamine, witch hazel, cashew oil from the shells, and, when less strong after a lapse of time, sweet briar. Some have considered the smell to be exceedingly unpleasant, others pleasant. However, as Eltringham (1925) pointed out, an odor which seems agreeable to one person may be obnoxious to another. For example, he himself did not like the scent of witch hazel, which is rated either "pleasant" or "not unpleasant" by all five of the present members of the Tropical Research staff.

The following account of the abdominal glands is derived from our Trinidad studies.

In *H. erato hydara* these glands in both sexes sometimes give off a strong odor which is practically never discernible in the course of courtship, and never at the roost or in social chasing. To us it always resembles witch hazel in the living or freshly dead insect. As noted by previous observers of this and other subspecies

(Longfield, 1926; Collenette, 1929), the odor is stronger and occurs more frequently in females than in males. In the Trinidad form it appears in the female only after mating; it can be detected as a faint fragrance, similar to that of the wings (see below), almost immediately after copulation. It then develops slowly, becoming strongest in the midst of the oviposition period, then declines and has not been detected after the thirtieth day of the imaginal period. It does not deter either subsequent courtings or at least one additional mating. It never develops in unmated females, even after death; however, it may be strong in the glands, excised and bottled, of males and mated females for many weeks although, as will be discussed below, it alters its witch-hazel-like characteristics. The odor can only rarely be detected in living males of any age; those which emit the odor in response to seizures are usually moderately but not extremely young.

The abdominal glands are everted in apparent defense only when the insect is seized either near the base of the wings, or by the head, thorax or abdomen. The seizure may be by any means, whether a natural enemy, fingers or forceps. Grasping the tips of the appendages, or simply touching or tapping any part of the insect, does not evoke the response. Neither does a purely visual stimulus, or the odor of a lizard's cage. Also, as previously stated, it is a rather uncommon response, being fairly predictable only in moderately young, mated females, or when the abdomens of either sex are squeezed so forcibly that the extrusion may be considered a purely mechanical effect. It is only with the stronger stimuli that the "stink clubs" (p. 177) are exserted.

On the other hand, the ventral curving of the abdomen, which always accompanies extrusion of the gland, occurs to a greater or lesser extent, and without extrusion, whenever the insect is seized as described above. It will be noted that this abdominal curving-under is in the opposite direction from the upward tilting of the female abdomen when the gland is everted during courtship (p. 177). The restricted use of the gland in defense is true of field-caught as well as insectary-reared specimens, although it may be that male responses are even rarer in these "tame" butterflies. However, insectary females of appropriate age respond fully to seizure, and evert the glands even late in life, when no odor can be detected.

Dr. Eltringham's (1925, 1926) dissections and serial sections of the abdominal glands in the female of this species and subspecies showed only a single pair of glands, although there were two pairs in related genera. It seems likely therefore that the protective function, if any, of the strong odors evolved directly out of the sexual function, and that their chemical composition is not necessarily different from the substance(s) obviously used in courtship but not detectable to the human sense of smell. The actual courting use of the glands in the female, apparently reported for the first time in the present study, confirms Eltringham's surmise concerning such a function in addition to that of defense.

In comparison with *H. besckei* in Brazil (Müller, 1877, 1912), *H. erato* has this defense mechanism much less well developed. It may be that *H. erato* represents a preliminary stage in evolution, in which the everting of the glands when the insect is prevented from escape, and the concomitant discharge of abundant odoriferous material, is scarcely more than displacement behavior in which a sexual response is given. In other species of the group it has probably developed into a highly evolved defense. According to Müller it was a habitual defense response with both sexes.

Mr. Ernest C. Crocker of the Flavor Laboratory of Arthur D. Little, Inc., in Cambridge, Mass., has most kindly given his impressions on samples sent him of the harpes of a 72-hour-old male and the abdominal scent gland of a 16-hour-old, mated female. Mr. Crocker and two assistants examined the material within five to six days after the specimens were killed and sent to him in small vials, via airmail. He writes as follows (personal communication): "The . . . . *Heliconius erato* specimens are all animal-like, earthy, musty, and dulcy, and yet somewhat flowery. Their distinctive features follow . . .

"The harpes, 72 hours old, have distinctly phenyl carbylamine character (see next description).

"The scent glands ♀, 16 hours old, have particularly sharp, strong phenylcarbylamine (phenyl isocyanide, C_6H_5—N=C type) odor. This odor also suggests styrene (phenyl ethylene) and phenyl proprionaldehyde. Enclosed is some of that aldehyde and also some brom-styrene. In our opinion, this insect odor must be due to a phenyl compound, with no more than 2 or 3 carbons on the side chain and possibly an oxygen or nitrogen."

The odors of the samples, when compared with living specimens in Trinidad, did not bear any obvious similarity to those of the abdominal glands of either sex; however, within a few days the odor of the excised glands decidedly resembled the brom-styrene sample.

2. *Scent Scales.* As in other species of the genus (Müller, *loc. cit.*), specialized scent scales are present in the male only, and only on the friction surface of the anterior portion of the

upper hindwing. In the present species they are small, dark, and mostly concealed by the larger uncolored scales of the friction surface. Although they apparently play a definite role in courtship (p. 179), their odor is only rarely and questionably detectable in courting males. In fresh or long-preserved friction surfaces, however, the odor is moderately strong, and to us appears decidedly flowerlike. Mr. Crocker examined samples of this area, from the 72-hour-old male mentioned above, and noted that the general character was that of the abdominal glands, namely "animal-like, earthy, musty and dulcy, and yet somewhat flowery." In distinctive features they were "tobacco-like, somewhat fecal (scatole) and have some finnanhaddick amine character. No carbylamine-like odor noted."

3. *Odor of Thorax and Wings.* A fragrance, apparently distinguished here for the first time, is clearly perceptible in both sexes on the dorsal side of the thorax and on the upper surfaces of all the wings, especially basally. To our perception it is not distinct from the fragrance of the scent scales proper, except that it is weaker. In dried specimens or detached wings, even many weeks after death, there may be, as in the abdominal glands, a resemblance to the odor of brom-styrene. In living butterflies this fragrance does not become apparent until about the second day in females and the third in males. The odor does not depend on mating or even courting to be evident in females; therefore at least in these females it cannot be merely the result of fanning the products of the scent scales by courting males.

4. *Odor of Young Imagos.* A musky odor is evident in very young imagos; usually it is not detectable after about the third day. It has not been definitely localized except that rarely it has seemed strongest at the tip of the female abdomen.

To summarize: Three or four distinct odors are discernible to human beings in *H. erato;* at least two of them are probably involved in defense. First, the abdominal glands emit an odor which, in the intensity detectable to human beings, is at least indirectly a product of the male harpes; it reaches its maximum development in mated females. It is a phenyl compound which changes, for human sense, from a witch-hazel-like to a phenyl-carbylamine-type odor within a few days. The odor which almost certainly is emitted by the extruded gland of unmated females during courtship is not detectable by the human sense of smell. Second, as in other members of the genus, fragrant scent scales are present on the anterior upper portion of the male hindwing. Third, a fragrance, apparently similar both to that of the male scales and, faintly, to that of the abdominal glands, develops on the thorax and upper wing surfaces of both sexes, mated and unmated. Fourth, a musky odor is apparent only in recently emerged imagos, especially females, and is possibly strongest at the distal end of the abdomen.

Any defensive use of the witch-hazel-like odor of the abdominal glands seems to be quite obviously a secondary development from an originally sexual function. A puzzling point is that the odor reaches maximum strength in ovigerous females. Since these are biologically the most valuable members of the species, it is possible that their increased odor has indeed some selective significance. However individuals of this age do not seem to be more resolutely refused by predators than are others of the species.

It seems that the basis of the aposematic taste of these butterflies may very likely be a secretion of the thorax, or of the thorax and the wing bases, which may be also responsible for the fragrance of these regions in both sexes. Perhaps it is contained in the weather-proof oily coating of the scales. The odor probably also has a sexual, or at least a social significance within the species.

The function of the male scent scales is probably sexual only. The musky odor of young adults may well be of value both socially, sexually and aposematically.

An interesting related question is that of the general function of odor as an aposematic signal in various Lepidoptera. Some authors have remarked both on this protection and, simultaneously, on the avian beak marks which often show on the wings of living butterflies, and so indicate successful past escapes. Assuredly birds must be the chief natural enemy of adult diurnal Lepidoptera. Yet these vertebrates, according to the best current knowledge, cannot smell. The chemical source of the nauseous taste must therefore extend to the insects' wings' themselves, being perhaps contained, as suggested above, in the oily scale coating. The odor(s) of the insect, as opposed to the taste, must be chiefly of use in deterring reptilian and mammalian enemies.

It also seems clear, as suggested by Jones (1930, 1931), to anyone who has observed sleeping heliconiids in the field, that the protection afforded by roosting aggregations is that of reinforced odor rather than of conspicuous warning color. Roosting heliconiids, of whatever species, are exceedingly inconspicuous objects even in daylight.

G. Oviposition. The foodplant of *H. erato hydara* in Trinidad is *Passiflora tuberosa* (Jac-

quin). Eggs are laid only on the youngest shoots and tendrils, generally one to a plant. The vines seldom grow taller than six feet, each shoot dying back after flowering and the young shoots tending to come from the ground or near it. Hence laying females usually fly only a few inches or several feet above the ground. Most eggs are laid between noon and 2:30 PM, which is otherwise a period of relative quiescence for the species. One egg, however, was laid at 8:45 in the morning and another as late as 3:30 PM. The first egg normally appears on the eleventh or twelfth day after emergence, although a single individual, mated with a Surinam male 45 minutes after emergence, laid the first egg on the fourth day; another female commenced laying on the tenth. In no other females, among more than 20 recorded layers of known age, was an egg laid earlier than the eleventh day. Egg-laying continues at the rate of 0 to 4 eggs, very rarely 6, daily for about two weeks or slightly more, until a total of up to about 24 eggs have been deposited. A typical female laid 18 eggs in 15 days, with a final gap of four days between the seventeenth and eighteenth egg. The higher numbers, two to four a day, are laid in the first half of the period.

H. Longevity. Under ideal conditions, males usually live in the insectaries slightly more than a month after emergence although rare individuals live more than two months. Females habitually live six weeks or more, again if no untoward circumstances, such as long rainy or windy spells, occur. The record for any individual was 91 days in the imaginal state, attained by a female reared in captivity. She had been mated on her third day and laid about 20 eggs on schedule. Counting the total 26 to 30 days of the developmental stages, this gives a record lifespan of about four months.

VI. Experimental Analysis of Social Behavior

A. Introduction. Experimental work was undertaken in order to determine the releasing mechanisms of courtship and other types of social behavior. In particular it was desired to discover whether any role was played by color in these activities, and if so, whether it was as an innate or acquired response.

The evidence was accumulated in three ways: first, through concealing the female so that only her odor could guide the male, the sense of sight being altogether or partially eliminated. Second, the color and pattern of both sexes were changed in various ways, in order to establish the relative importance, if any, of hue, brightness and markings. Third, the responses of both sexes were tested with models.

B. Work with Concealed Females. 1. *Method.* Six virgin females, each between 24 and 72 hours after emergence, were placed, singly, in a box 2 × 2 inches in size, which gave them sufficient space in which to move their wings freely. In the first tests the cardboard covering top and bottom was punctured with about a dozen nailholes, through which the female was quite invisible; in other tests the top was covered with khaki-colored mosquito netting, which presumably made the odor of the female more discernible, but through which she was dimly visible to the human observer. Each of the butterflies, before being placed in the box, had been tested with one or more young males and found to be normally attractive—that is, she elicited Stage I courting behavior.

2. *Results.* When the box with an enclosed female was left on a stool in the insectary, in the path of freely flying males, none ever dipped toward it, or paid it any other evidence of attention, whether the box was covered with punctured cardboard or netting. Similarly, virgin females kept in a cage 18 × 18 × 12 inches, with one of the long sides covered with wire netting, did not attract the attention of males. It is concluded that female odor alone is not sufficient in this species to inaugurate courtship.

C. Work with Painted Butterflies. 1. *Method.* A variety of unsuccessful preliminary attempts was made to change the color of a living butterfly. Sometimes the color, such as water color or India ink, would not adhere to the oily surface of the scales. Removal of the film with weak acid damaged the wing, as did bleaches strong enough to fade out the black pigment. Painted tissue attached to the wing with an appropriate cement was so heavy that the butterfly could not fly. Certain paints, such as artists' oils, did not dry fast enough. Finally, the butterfly was often so badly shocked by the procedure—apparently because of the necessary handling—that it never recovered normal activity levels and died more or less promptly.

The following technique, however, was slowly perfected and can now be recommended (Pl. III, Fig. 15). The main precautions to be observed are to work slowly and to touch the butterflies with the fingers as little as possible. Properly done, no manual handling whatever is necessary, after the butterfly has been placed in the envelope, and hardly any touching with forceps. Stroking with the paint brush should be feather-light.

Step. 1. The butterfly is placed in a glassine envelope, the wings folded over the back, as

soon as captured, either in the field or the insectary; handling and warming are avoided. With specimens taken from the insectary, capture and painting is done with the least shock at night, the insect being taken from the roost. After painting it can frequently be rehung in approximately the same place.

Step 2. The envelope is placed in a flat wooden box about 3 × 3 × 1 inches, with a glass bottom. The box is turned upside down so that the butterfly can be observed through the glass. Through one side of the box enters the nozzle of a short tube attached at the other end to a small fire extinguisher or bicycle pump filled with carbon dioxide. The stopcock of the cylinder has previously been turned on and the gas regulated to a very low rate of flow. The butterfly in the envelope is left in the box for three to five minutes, or until a minute or two after all movement has stopped. If the butterfly becomes active during painting, it can be further anaesthetized merely by placing the glass-bottomed box briefly over the insect on the spreading board (see below). Deep anaesthetization should be avoided, since butterflies so treated do not always regain their normal vigor, and may not court.

Step. 3. A piece of oiled paper is laid across the slot and breadth of the butterfly spreading board, to support the body in a sling and protect the wings from the wood. The butterfly is shaken onto the paper and, with the wings remaining folded, fastened into place with two strips of paper pinned across, but not through the wings, in such a way that the area of underwing to be painted remains uncoverd.

Step 4. With a fine paint brush ether is brushed lightly several times across the area to be painted.

Step 5. The underwings of one side are painted as desired with a waterproof, fast-drying lacquer. In the present experiments, "Flopaque" was used (manufactured by Floquil Products, Inc., Cobleskill, New York). The paint should be applied thinly and restroking avoided.

Step 6. Papers are removed, the butterfly flipped over, and the underwings of the other side similarly treated.

Step 7. By careful manipulation of paper strips, the butterfly's wings are now opened and fastened to the board, without the touch of fingers or forceps. Extreme care must be used not to exert pressure on head or body by pinning their paper covering too tightly or by letting the wax paper sling under the body become taut. A strip loosely covering eyes and body, however, helps keep the insect quiet when it starts to emerge from the anaesthetic. Brushing with ether and painting then proceeds as on the underwings.

Step 8. After painting is completed the setting board is taken to the insectary and the butterfly released by unpinning the paper strips, thus avoiding further handling. When the procedure is properly carried out, the insect is usually capable of flying at once to a resting place. When the painting has been done at night, the butterfly can often be simply rehung with forceps on the roost. In any case, the butterfly usually remains quiescent for some hours, although females give normal courting responses sooner. Recovery should be altogether complete by the next day. When possible, butterflies should not be painted sooner than 48 hours out of the chrysalid, since the wings of freshly emerged butterflies are very easily damaged and the insect is more subject to after-effects of the treatment. However, for special procedures employed to ensure a butterfly's being unconditioned to the color "red," see p. 187.

If the use of other types of paints is desirable, such as fluorescent paints, or water colors with particular spectral characteristics, they may sometimes be successfully applied as follows: the coloring matter is painted on a sheet of lens tissue and allowed to dry, in several coats if necessary, so that the tissue becomes opaque. The paper is then cut in pieces of the desired size and shape. The butterfly is anaesthetized and placed on the spreading board as for painting. The painted bits of tissue are fastened in place with rubber cement diluted with xylene. The method is only rarely satisfactory, since the extra weight often prevents the butterfly from flying well. No successful attempts have been made to remove the insects' scales before attaching the paper, and so reducing the weight of paint needed to efface the natural color; the wing tissue is always too much weakened either by the use of chemicals or by stripping with Scotch tape.

Table 4 gives a summary of the various color and pattern changes effected; Table 1 (p. 172) shows a spectrophotometric analysis of the paints used.

No positive responses are included where there was danger of the female's showing effect of summation. The experiments were conducted both with individuals which had emerged and been kept in isolation, out of sight of their own species, and not allowed any sight of the color red, and those which had not been so isolated (see p. 187).

2. *Results.* In brief the following principles may be stated, concerning the social responses to painted butterflies.

a. *Courtship.* These responses are given in summary in Table 4. Positive responses were counted for the sex involved if the female, when approached or actively courted by a painted male, elevated the abdomen, extruded the yellow organs, apposed the forewings and vibrated the hindwings. If only the abdomen was elevated and the yellow organs extruded, a half-response was counted (the hindwings were never fluttered without the abdominal response). The very rudimentary response of merely raising the abdomen slightly, without extruding the yellow glands, was not counted, since this is sometimes done when the insect is resting alone, or feeding.

Positive responses were counted for males to painted females if he approached the female and made repeated, continuous courting dashes at her, in at least the typical Stage I rear position (p. 177).

It will be seen from Table 5 that all changes of color and pattern attempted resulted in positive responses in at least one sex. What cannot be fairly indicated in the table, because of the difficulties of the procedure and the many variables of weather, physiological state of the butterflies and so on, is the relative popularity of the various colors. This can be recorded only in general terms, resulting from the author's prolonged observation of painted butterflies over a three-season period. It was borne out in every respect in the amount of general social chasing of the variously painted individuals by other members of the group after the active breeding age was passed (p. 180).

Briefly, the farther the altered color of the forewing pattern is distant from the normal scarlet in the spectrum, the less notice is taken of the butterfly, either by the opposite sex or as a subject for general social chases. That is, butterflies with the forewing band painted orange or yellow were almost or quite as successful socially as butterflies repainted in natural colors. In most trials the orange was more successful than yellow, that is, more promptly responded to by members of the opposite sex, and slightly more subject to social chasing than yellow. Yellow-green bands were less successful, while greens, blues, violet, positively ultraviolet white and all black were least so. Negatively ultraviolet white was, again, moderately successful, but since for this color Chinese white (zinc oxide) water color on bits of tissue had to be used, the butterflies were both overburdened and scarcely weather-proof; hence their general social life when fitted with this color was not subject to valid comparisons.

All-black butterflies and those with inconspicuous short-wave markings, such as all black with dull green radiations on the hindwings, were notably unsuccessful. In fact no completely black female, with even the pinkish underband of the forewing eliminated, ever drew a positive response from a male; the one case of mating given in the table was a young female which had been given only a thin wash of black, permitting a pinkish tinge to show both above and below. Out of eight individuals painted more or less black, this was the only positive response. Black females were repeatedly ignored, even though, at the close passing of males, all the usual Stage I responses were given: apposed forewings, gland extruded from the elevated abdomen and vibrated hindwings. Occasionally these unnoticed females even chased after such passing males, yet never attracted more than the briefest dips in their direction.

b. *Social Chasing.* Behavior toward males and non-receptive females which had been painted, paralleled that of courting individuals. The farther away the spectral reflectance of the paint used from the orange-red region, the less was the butterfly chased. This was regardless of brightness: pale blues, greens or green yellows were disregarded in comparison with deep oranges and reds. Grays were treated like black. Often an ignored painted female would chase other butterflies, which even then would only rarely take any apparent notice of it. There was no reciprocal circling. Blue-marked butterflies, which often lived weeks after painting, were ignored almost or quite as completely as all-black individuals.

c. *Roosting.* In this activity the painting of individuals made no apparent difference. All were accepted on the roost, and made and held for themselves positions in close juxtaposition both to normal butterflies and to those otherwise colored.

D. Work with Butterfly Models. 1. *Method.* The most successful models were made of heavy black or colored cotton felt or canvas cut roughly to the shape and size of a *Heliconius erato.* To these were fasted bits of colored paper in various sizes and patterns. Along the longitudinal axis of the "body" of each model was threaded a black insect pin. The other essential part of the apparatus was a flexible 2½-foot wand of split bamboo. To the small end of the wand was attached a small magnet, such as is used for fastening notes to bulletin boards. A petroleum-base adhesive attached the magnet to the bamboo, both magnet and wand being painted dark green and black. By means of the magnet and the insect pin through the body of each model, a series of different models could be presented to a butterfly in as rapid succession as desired.

TABLE 5. ALTERATIONS EFFECTED IN COLOR OF LIVING *Heliconius erato hydara.*

Each color change indicated by "X" was effected in at least one butterfly of each sex. The responding individual, painted or not, had not been allowed previously to see the color red, except that of its own wings. Positive courting responses, sometimes including copulation, were obtained at least once for each color change effected in each sex. However, responses were exceedingly rare, and almost always weak to the blues, greens and blacks, while oranges and yellows were treated almost like normally colored butterflies. Change in pattern was of relatively little importance. Females were in general less influenced by color change than were males. Numbers in parentheses after color names refer to paints used (For spectrophotometric analyses, see Table 1, p. 172).

		No Color Added	Red (31)	Orange (25)	Yellow (20)	Green (12)	Blue (5)	Blue-green (10)	White (UV Positive)	White (Zinc Oxide)	Pink (36)
Red forewing band unaltered	Hindwing with spot		X					X			
	Hindwind with radiations		X				X				
	Under fore- and/or hindwing with spots, crosses, dots, in many combinations (for identification)		X	X	X	X	X	X	X		
Forewing band altered in color (upper & underwings)	Hindwing unaltered			X	X	X	X	X	X	X	X
	Hindwing with spot of same color			X	X			X			
	Hindwing with radiations added				X						
Upper forewing band altered in color, blackened on underwing	Hindwing unaltered			X				X	X		
Upper forewing band blackened	Underforewing band painted								X		
	Underforewing band unaltered (pale pink, pos. UV)	X									
Forewing band blackened on upper & underwings	Hindwing unaltered	X									
	Forewing with colored spot, hindwind unaltered		X								
	Hindwing with spot				X				X		X
	Hindwing with radiations					X	X				
	Fore & hindwing with single spots				X						
Upper forewing band altered in color, underforewing band painted white (pos. UV)				X				X	X		

The most successful, and in general the only successful technique, consisted in jiggling the wand gently up and down near the butterfly to be tested by holding the wand in the left hand and tapping it rapidly near the base with the right forefinger. This caused the model at the tip to flutter up and down in a fairly realistic way. When models made entirely of paper had to be used (because of desired spectrophotometric characters), an underlayer of flapping black felt beneath the stiff paper gave the air current which is an important factor in the species' courtship pattern.

As in the work with paints, spectrophotometric reflectance curves were secured for the colored textiles and papers used (Table 1, p. 172).

The experiments were conducted in three groups. First, with individuals which had not been kept isolated, after their emergence, from normally colored members of their species, and which hence might have become conditioned to the color "red." Second, with individuals which had been kept in isolation from the time of their emergence until the hour of the experiments. These butterflies were not fed at all, or allowed to see any red object, even being isolated from other chrysalids, lest the red wing band which shows through the chrysalid on the last day before emergence should be seen by and possibly imprinted on an emerging neighbor. The isolated individual, after 24 to 48 hours, was then painted or not, depending on the needs of the subsequent experiment.

The isolation of unpainted examples was maintained as follows: The cages were kept at a distance from one another and from the insectaries, and the sides facing occupied cages were masked with branches. Wild *erato* almost never enter the garden where the small cages are kept, so there was very slight chance of conditioning by the brief sight of a passing member of the species.

The third group of test butterflies was even more rigidly guarded against possible conditioning to red. In this group the possibility was eliminated that a just-emerged butterfly might be conditioned or imprinted with the red color of its own wing markings, whether the scarlet forewing bands or even the tiny red dots and bar near the base of the underwings. It proved difficult to control this factor, but the following technique finally resulted in active, uninjured butterflies, fully protected against a previous sight of red, in about seventy-five per cent of the individuals treated.

Caterpillars of the imagos to be tested under these conditions were reared in complete isolation, as in the second group described above, and allowed to pupate hanging from a four-by-four inch piece of glass. On the night before emergence, the glass with its dangling chrysalid was placed over a hole in the lid of a light-tight shoebox. On top of the glass was placed a six-by-six-inch Corning Glass filter (Col. spec. 5-60, "H.R. Lantern Blue"); while transmitting the near-ultraviolet, violet and blue freely, it has a sharp cut-off in the green at 520 mμ. The filter was tightly sealed into place with black photo tape. Under this blue filter the test butterflies, in turn, emerged on schedule and were found on release to be less battered than those which emerged in complete darkness. Each individual, when examined the same evening under an identical blue filter fastened over an electric desk lamp, was found to be fully expanded with the wings relatively firm. The room was kept in complete darkness except for the blue light, so that the butterfly at no point had an opportunity to catch sight of its own scarlet markings. The butterfly was then removed from the box, anaesthetized with carbon dioxide and fastened on the spreading board, as in the usual painting technique (p. 183). The only difference was that after each wing surface had been arranged in position for painting, the eyes were covered closely with criss-crossed strips of black felt. This enabled actual painting to proceed under ordinary electric light, without the use of the blue filter. A blue paint, with relatively low reflectance in the long wave regions, was used (Sample 5, Table 1). After completion of the painting, the butterfly was hung in the insectary, from which all red or orange flowers had been removed, as well as all other butterflies. The young butterfly was allowed to feed well the following morning. Usually these butterflies which had emerged in a small box, been kept there all day and painted less than twelve hours after emergence were not in condition for testing until the day following feeding, that is, 48 hours after emergence. One did not become fully active until his seventh day, when he was successfully tested. The mortality is in any case high from this rough treatment, but the results, from the seven specimens which survived it, seem conclusively to be based on innate, and not learned or imprinted, behavior. These results (Table 6) agree extremely well with those obtained for the more numerous individuals tested in the other two groups, in which previous exposure to red was either partially or not at all controlled.

Throughout the experiments in all three groups, no positive responses were counted where there was danger of the female's showing summation, or heterogeneous summation. A model, normally unsuccessful, such as plain

TABLE 6. RESPONSES OF BLUE-PAINTED *H. erato hydara*, REARED IN ISOLATION, TO MODEL BUTTERFLIES

Part a: Field Data

Explanation: Each of the 7 specimens was maintained in isolation, without exposure to orange-red or red, until the time of the test. See text, p. 187 ff. All tests were conducted in calm, sunny weather in the large insectary. Each model (p. 185, Text-fig. 2, Pl. III, Fig. 17) was presented for a maximum of 30 seconds. A response, if any, usually occurred within five seconds. In males, a single slight dip toward the model was counted as a "minimum" response, several dips or short chases as "good," and definite Stage 1 courtship, with persistent pursuit, as "strong." The single female's responses were similarly gauged from the strength of her courting behavior, shown by the degree of abdomen elevation, extrusion of yellow gland, apposition of forewings and fluttering of hindwings. Color sample numbers refer to spectrophotometric analyses in Table 1, p. 172.

Individual No.	Presentation Order	Color Sample No.	Model Type	Model Hue	Response
1(♂)	1	4	2 colored bands on black	Blue	None
	2	8	"	Blue-green	None
	3	15	"	Yellow-green	None
	4	28	"	Orange-red	Good
	5	17	"	Yellow	None
	6	22	"	Yellow-orange	Minimum
	7	9	"	Blue-green	None
	8	33	Solid color	Red	Good
	9	—	"	Black	None
	10	23	2 colored bands on black	Orange	Good
2(♂)	1	4	2 colored bands on black	Blue	Minimum
	2	15	"	Yellow-green	None
	3	17	"	Yellow	Minimum
	4	8	"	Blue	Minimum
	5	28	"	Orange-red	Strong
	6	22	"	Yellow	Minimum
	7	33	Solid color	Red	Strong
	8	4	2 colored bands on black	Blue	Minimum
	9	23	"	Orange	Good
	10	8	"	Blue	None
	11	29	"	Red	Strong
3(♂)	1	7	Solid color	Blue	None
	2	14	"	Green	None
	3	18	"	Yellow	None
	4	27	"	Orange	Good
	5	7	"	Blue	None
	6	33	"	Red	Good
	7	36	"	Pink	Minimum
	8	22	"	Yellow	None
	9	33	"	Red	Strong
	10	4	2 colored bands on black	Blue	Minimum
	11	16	"	Green-yellow	None
	12	17	"	Yellow	None
	13	22	"	Yellow	Minimum
	14	8	"	Blue	Minimum
	15	15	"	Yellow-green	None
	16	23	"	Orange	Good
	17	16	"	+UV White	Minimum
	18	4+28	2 colored bands on color	Orange-red on blue	None
	19	28+22	Colored stripes on black (Text-fig. 2c)	Red; Yellow	Minimum
	20	—	2 colored bands on black	Zinc oxide white	None
	21	28	"	Orange-red	Strong
	22	16	"	Green-yellow	None
	23	34	"	Violet-red	Strong

TABLE 6. *(Continued)*

4(♂)	1	7	Solid color	Blue	None
	2	11	"	Blue-green	Minimum
	3	18	"	Yellow	Minimum
	4	27	"	Orange	Strong
	5	7	"	Blue	None
	6	33	"	Red	Strong
	7	4	"	Blue	None
	8	17	"	Yellow	None
	9	22	"	Yellow	None
	10	28	"	Orange-red	Strong
	11	9	"	Blue-green	None
	12	36	"	Pink	Good
	13	17	Black stripes on color (Text-fig. 2d)	Yellow	None
	14	1	Solid color	Violet	None
	15	16	2 colored bands on black	Green-yellow	None
	16	34	"	Violet-red	Good
	17	—	"	Zinc oxide white	Minimum
	18	28	"	Orange-red	Good
5(♂)	1	7	Solid color	Blue	None
	2	18	"	Yellow	None
	3	27	"	Orange	Strong
	4	14	"	Green	None
	5	33	"	Red	Strong
	6	18	"	Yellow	None
	7	28	2 colored bands on black	Orange-red	Strong
	8	22	"	Yellow	None
	9	33	Solid color	Red	Strong
	10	23	2 colored bands on black	Orange	Strong
	11	22	"	Yellow	Good
	12	16	"	Green-yellow	Minimum
	13	8	"	Blue	None
	14	33	"	Red	Good
6(♂)	1	7	Solid color	Blue	Minimum
	2	14	"	Green	None
	3	18	"	Yellow	None
	4	27	"	Orange	Good
	5	7	"	Blue	None
	6	36	"	Pink	Minimum
	7	33	"	Red	Strong
	8	4	2 colored bands on black	Blue	None
	9	—	"	+UV White	None
	10	22	"	Yellow	Good
	11	17	"	Yellow	None
	12	29	"	Red	Good
	13	7+28	2 colored bands on color	Orange-red on blue	None
	14	28	2 colored bands on black	Orange-red	Strong
	15	34	"	Violet-red	Good
	16	16	"	Green-yellow	None
	17	23	"	Orange	Strong
	18	33	Solid color	Red	Strong
7(♀)	1	33	Solid color	Red	Strong
	2	27	"	Orange	Strong
	3	7	"	Blue	None
	4	14	"	Green	None
	5	18	"	Yellow	None
	6	36	"	Pink	Minimum
	7	7	"	Blue	None
	8	27	"	Orange	Good
	9	33	"	Red	Strong

TABLE 6. *(Continued)*

10	22	2 colored bands on black	Yellow	Minimum
11	4	"	Blue	None
12	23	"	Orange	Minimum
13	17	"	Yellow	Minimum
14	–	"	Zinc Oxide White	Minimum
15	29	"	Red	Minimum
16	16	"	Yellow-green	Minimum
17	28	"	Orange-red	Good
18	33	Solid color	Red	Good
19	28+4	2 colored bands on color	Orange-red on blue	None
20	34	2 colored bands on black	Violet-red	Good

Part b: Summary

Color Sample No.	Hue	Responses*			
		None	Minimum	Good	Strong
–	+UV White	1	1	–	–
–	Zinc oxide White	1	2	–	–
1	Violet	1	–	–	–
4	Blue	4	3	–	–
7	Blue	8	1	–	–
8	Blue	3	2	–	–
9	Blue-green	2	–	–	–
11	Blue-green	–	1	–	–
14	Green	4	–	–	–
15	Yellow-green	3	–	–	–
16	Green-yellow	4	2	–	–
17	Yellow	4	2	–	–
18	Yellow	5	1	–	–
22	Yellow-orange	3	4	2	–
23	Orange	–	1	3	3
27	Orange	–	–	3	3
28	Orange-red**	–	–	3	5
29	Red	–	1	1	1
33	Red**	–	–	4	9
34	Violet-red	–	–	2	1
36	Violet-pink	–	3	2	–

*Black models with colored bands and solid colored models only.
**Used frequently, to check condition of specimen.

black felt or a stick, was used as a test in suspected cases. Contrariwise, a butterfly's threshold was gauged periodically during an experimental session by presenting a highly successful model. If a positive response was given to this, the insect was considered still to be in a mood adequate to continue the session.

In the entire series of tests, 53 young butterflies were used, including 30 males and 23 females. Of the total, 25 had not been isolated from their own species, 21 had been isolated from red except for the sight of their own scarlet wing band, and 7 were both isolated and painted blue.

2. *Results.* Since the factor of odor was removed, and movement controlled, the results were more clean-cut than in the case of painted specimens.

It was found through the use of models that both motion and hue are of importance in courtship. In order to elicit the hindwing flutter of females, the model had to be waggled up and

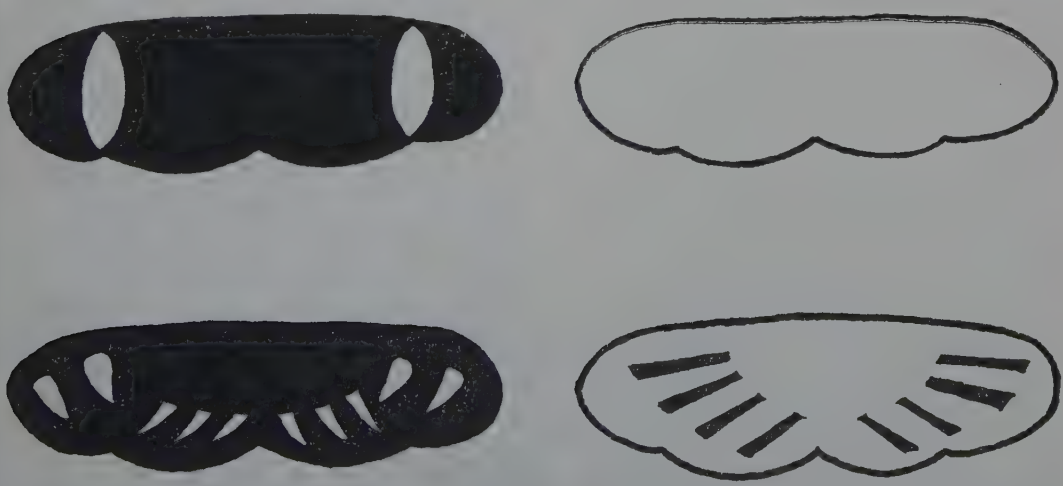
down immediately behind, but not touching, her hindwings; the determining factor in this stage was the air current thus formed. A model, otherwise highly successful, which was vibrated to one side or in front of her, as in later stages of courtship, did not cause her either to raise the abdomen, extrude the yellow gland, appose the forewings or flutter the hindwings.

The response of males to models was partly to the fluttering movement of the model. Motionless models, tied by black threads to twigs or leaves, or left on the ground, only very rarely elicited a dip from a passing male. Models fluttered on the wand, however, were often chased and more rarely drew the first stage of courtship, that is, repeated short dashes with the friction surfaces not exposed.

The various models themselves were successful in direct proportion to their similarity to the size and color of living butterflies, with one exception; the most successful model of all was made entirely of negatively ultraviolet red felt without any pattern whatsoever; in fact it acted as a supernormal stimulus, eliciting a response from either sex when not only all other models failed, but often when the threshold was so high that a living member of the opposite sex in an appropriate physiological state did not win a response. It was accordingly used to test the continued responsiveness of an individual from time to time during a series of tests, or to determine its threshold before an experimental session.

Models with forewing bands of naturalistic size and position but varying only in color were successful in about the same sequence as were butterflies with the bands painted: the most successful were the naturalistic orange-red. However, the red, orange and yellow-orange were almost equally successful, lemon yellow and greenish yellow notably less so, while greens, blues, violets and positively ultraviolet white least so. In general, the colors reflecting ultraviolet the most strongly were less popular in comparison with purer colors of the same general spectral region. Negatively ultraviolet white drew responses rather unpredictably; on the whole they were moderately successful with individuals of low threshold. No response was obtained to an all-black model except weakly, by several females of very low threshold which were already highly stimulated by preceding models or by actual courting. These individuals responded equally well at that stage to practically any small object moved close behind them, including dead twigs and the observer's finger. Pattern was of little importance, so long as the background was black. Text-fig. 2 shows the type of variations used. However—except for the solid color red models, approaching the natural color of the butterfly's band—solid colors, with or without contrasting or gray bands or other markings, were less successful than their counterparts in which the model was black with a band of unnatural color. For example, an all-yellow model was less successful than a black model with a yellow band. An all-orange model however usually elicited a strong positive response.

Models with a wingspread of from about two-thirds to one and a half times normal were of acceptable size; models notably smaller or larger than these were unsuccessful, regardless of color or the individual's threshold.



TEXT-FIG. 2. Examples of models used in butterfly courtship experiments. **A**, normal type: black with colored bands; **B**, solid color; **C**, colored stripes on black; **D**, black stripes on color. Natural size.

Table 6 gives the series of responses by the seven individuals in which all chance of conditioning to red was avoided. Because of the importance of the order of model presentation the full field data are given in Part a. In the summary of the table, Part b, it will be seen that the results follow closely those summarized in the text above for all the 53 young butterflies tested.

Models elicited a minimum amount of attention from old males and old females, and were disregarded by all when manipulated before the roost.

VII. Conclusions Concerning Social Behavior

The principal conclusions to be drawn from the preceding observations and experiments on the social behavior of *H. erato hydara* are as follows:

1. Motion, odor and hue are the most important factors in courtship, and play roles both in the males and females.

2. Minor elements are size, pattern and shape.

3. Motion is the single most nearly essential element; without it neither odor nor hue can elicit courtship, and even the combination is of no value except in cases of low-threshold males mating with nearly motionless, freshly emerged females.

4. Odors function importantly as releasers in both sexes, and are apparently essential for carrying courtship to completion: no male ever tried to copulate with a model and the hindwing-flutter-elevated-abdomen response of females to models was always of short duration. Part of this, however, may have been due to the fact that the motions of the models were necessarily only rough approximations of the flutterings of live butterflies. On the other hand, motion and odor without color badges practically never elicit courtship in the male and odor alone never does. Females, however, quite readily accept courting males lacking in or different in color, providing the other elements in the pattern are represented and the female threshold is very low. Odor is certainly the primary stimulus in roosting, as it is in the non-social activity of oviposition.

5. Color badges act rather as a directive stimulus, or as a preliminary releaser at most; however in this role they are important to both sexes. Although under low threshold conditions either sex will respond with the first stage of courtship to models or individuals marked with practically any color, the most successful are those most nearly approaching the natural black marked with orange-red. The striking exception is the high sucess of all-red models, which, combined with the unimportance of pattern, indicates that the hue "red" itself is a decided releaser. (cf. the discovery of Tinbergen *et al.* (1941) concerning the supernormal black model in the grayling butterfly). The least successful of both models and painted individuals are those marked or solidly colored with hues reflecting mostly in the short-wave end of the spectrum, or colored very dark or black. Negatively ultraviolet white is rather unpredictable in eliciting responses. As might be expected, the toleration for unnaturally painted but living butterflies is wider than for odorless, clumsily moved models.

The conditions of the experimental work show that this preference for orange-red and near-by similar colors is distinctly innate, and that it is not a question of mere visibility: *H. erato* will come to paper flowers of any color except green and probe accurately. Living blue flowers of certain species, such as vervain, are visited freely, especially in the absence of more favored species. The color preference in feeding tests is distinctly for yellow first and orange second, in contrast to the species attraction for orange-red first, then orange and red, then yellow.

There was no apparent difference in social responses of butterflies conditioned to red by the sight of flowers, other individuals, or simply to their own wing colors, and those which had had no previous experience of red.

The red was of similar value in "social chasing" as in courtship, but of none in roosting.

It must be kept in mind that the "red" of *erato* is a nearly pure orange-red: its spectrum shows exceedingly low to negative reflectance in the ultraviolet, violet, blue, blue-green and green, very little in the yellow, and relatively strong in the orange and red, from 600 mμ up (Crane, 1954, p. 97, text-fig. 9a). Therefore, even if these butterflies prove to have, like bees and other insects, very weak visual sensitivity above 650 mμ, their perception of the orange region would still be adequate to make the orange components of their bands easily visible to them. The low reflectance of these bands in the yellow presumably would differentiate them adequately to the butterfly from the color appearing "orange" to human eyes; this orange, in the test material used, included moderately high reflectance in the yellow and sometimes in the yellow-green, as well as in both orange and red. It will be remembered that the color orange is not quite as attractive in *erato* courtship as a natural *erato* orange-red. However, until electroretinograms can be made of the butterflies' eyes, there seems to be no satisfactory method of determining the limits of their spectral sensitivity.

Perhaps the most interesting aspects of the results obtained are the following:

First, the selection of "red" as a releaser in courtship and social chasing indicates a secondarily evolved use of aposematic coloring. It is assumed here that aposematism in this and other red-and-black heliconiids, and their correlated development of Müllerian mimicry, occurred in evolution before the development of red as a social releaser. This is considered a likely assumption because of the widespread development of aposematism in the family. The members of the family show a variety of striking colors and patterns which very frequently do not include red, but rather browns and yellows all characterized by the inclusion of yellow and green, in addition to orange and red, in their spectral reflectances.

Second, both color pattern and secondary colors in addition to red, lack importance in social behavior, although black as a background has value. This is to be expected in a species which in other parts of its range, e.g. Surinam, is subject to conspicuous variations in pattern (Beebe, 1955).

The vitally interesting questions concerning the functions and origins of the various behavior elements, and of the relation of courtship and social behavior to phylogeny in butterflies, are left for a subsequent paper. It concerns comparative behavior in a number of species of heliconiids and is now in preparation.

VIII. Summary

1. Methods of rearing and maintaining broods of the butterfly, *Heliconius erato hydara,* are described which yield healthy adults suitable for observation and experiment. The caterpillars are reared singly and fed on *Passiflora tuberosa.* The adults are maintained in large, open-air insectaries.

2. It was established by behavior responses to filtered light that *H. erato* and a number of other butterfly genera are visually sensitive to light from at least 366 mμ to at least 600 mμ.

3. Experiments involving the use of colored paper flowers of known spectral reflectances among a full series of gray models established the existence of color discrimination in *Heliconius erato.* Models representing all spectral regions as well as negatively ultraviolet white (zinc oxide) were distinguished from all grays. Yellow is the preferred color in feeding responses, and the preference appears to be innate.

4. The general habits of flight, feeding and roosting of the butterfly are described. Daily activity is governed by temperature in the early morning and by the reduction of daylight in the afternoon. As usual in butterflies, the highest activity occurs on sunny days. Feeding is most prevalent during the morning, courting in the late morning and after 2:30 P.M. Egg-laying usually occurs around noon. These *Heliconius* are entirely flower-feeders, preferring yellow and negatively ultraviolet white blossoms; a few blues are visited; reds very rarely.

5. Aposematism and defense through unpleasant odor and taste are briefly discussed.

6. Social behavior is of three types—courting, social chasing and roosting.

a. Courting depends in both sexes on both visual and scent cues. The most important visual stimuli are motion and color. Motion releasers include, in the female, air currents made by the fanning wings of the male. Color approaching in hue the orange-red of the butterfly's forewing band is an important releaser in both sexes. Minor visual releasers are form, size and pattern. At least several odors are involved, emanating at least from a special yellow gland in the tip of the female abdomen and from scent scales on the anterior margins of the male hind wings. A more general body odor is also important. Although all the releasers are mutually dependent components of the courtship pattern, motion is the one most nearly indispensable. Visual cues are more important in the early stages, odor in the later ones. Courtship cannot be initiated by odor stimuli alone.

b. Experiments with painted living butterflies and with artificial models all underline the fact that the purer orange-reds are important releasers in both sexes. A solid-color orange-red model of normal size acts as a supernormal stimulus. The farther a color lies from this region in the spectrum, the less strong is usually the response. Greens, however, are even less popular than blues. This responsiveness to orange-reds is unquestionably innate.

c. Ultraviolet appears to be of no importance in the life of this butterfly, except to the extent that its absence (e.g. in negatively ultraviolet white) affects the perception of a color for the butterfly.

d. Social chasing is common, especially among aged individuals of both sexes. Color preferences are equivalent to those characteristic of courting. No evidence of intermale threat display or of territoriality was found. Territoriality may, however, prove to occur in wild populations.

e. Odor is of great importance, color apparently of none, in roosting behavior.

f. Evidence of displacement behavior is found in atypical courting, where the proboscis is extruded.

g. It is also suggested that *H. erato,* in which the apparently aposematic scent of females de-

velops only after mating, is evidence that the protective function of this odor developed out of a sexual function. The role of red as a sexual releaser, however, is held to be a secondary development from its original role in warning coloration.

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EXPLANATION OF THE PLATES

(Photographs by Rosemary Kenedy)

Plate I

Courtship of *Heliconius erato hydara*: Sequence of Stages (Photographed at 1/2000 sec.)

Fig. 1. Stage I. Male approaches resting female from rear.

Fig. 2. Stage I (cont.). By rapid fluttering of wings, male sends current of air against female.

Fig. 3. Stage I (cont.). Male backs off for another forward dart. Female shows beginning of response by starting to flatten hindwings. Meanwhile her abdomen is erected, the scent gland extruded from the penultimate segment and the forewings held apposed (see Plate II, Fig. 10). Male's partly uncoiled proboscis is unusual in normal courtship (cf. Plate II, Figs. 13, 14).

Fig. 4. Stage I (cont.). Male again flutters wings against female; sometimes, as in this picture, actually touching her wings with his own.

Fig. 5. Stage II. Male moves above and toward front of female; now the scent scales on the usually overlapping margins of his fore- and hindwings are uncovered.

Fig. 6. Stage II (cont.). Note outward expansion of female hindwings, and her partly depressed antennae.

Fig. 7. Stage III. Male prepares to alight beside female. Note elevated female abdomen with extruded scent organ visible as pale spot at tip.

Fig. 8. Stage III (cont.). This is followed by copulation (see Plate III, Fig. 16).

Plate II

Heliconius erato hydara, courtship: Details, and atypical courtship behavior.

Fig. 9. Female (right) giving partial response to courting male, Stage I: Her abdomen is elevated, the scent gland extruded and hindwings lowered and vibrated; the forewings however, are not apposed (cf. Fig. 10) (1/2000 sec.).

Fig. 10. Female giving practically full response: Characteristics of Fig. 9, plus apposition of forewings (1/2000 sec.).

Fig. 11. Male (upper) entering Stage II, showing exposed end of silver friction surfaces, bearing invisible scent scales on anterior margin of upper hindwings. Female is at left, back to camera, abdomen slightly elevated (1/2000 sec.).

Fig. 12. Courtship, Stage II, photographed by slow flash (1/200 sec.), indicating speed of male motion. Note that speed almost stops fluttering of female hindwings.

Fig. 13. Atypical courtship behavior: Male in final stages of courtship, never completed, palpates female head, thorax and forewings with his forelegs and uncoiled proboscis (1/2000 sec.).

Fig. 14. Atypical courtship behavior: Male alights in front of female, facing her, palpating her at intervals as above. Female is making partial courting responses, the scent gland partially extruded, hindwings slightly opened, forewings not apposed (1/2000 sec.).

Plate III

Fig. 15. Setup for painting wings of living butterfly: Left, carbon dioxide cylinder with glass-topped box for anaesthetizing insect. Center, spreading board with butterfly held in place by strips of paper. Background and to right, fast-drying lacquers, solvent, and brushes for painting; ether is used for partially removing oily coating of scales before painting.

Fig. 16. A male butterfly with forewing band painted blue above, black below, copulating with normally colored female.

Fig. 17. All-red felt model butterfly being attached by insect pin to magnet on end of split bamboo wand.

Fig. 18. Black felt model with scarlet paper spots fastened to wand, being fluttered behind female, to simulate Stage I of normal male courtship (1/2000 sec.).

FIG. 1

FIG. 2

FIG. 3

FIG. 4

FIG. 5

FIG. 6

FIG. 7

FIG. 8

IMAGINAL BEHAVIOR OF A TRINIDAD BUTTERFLY, HELICONIUS ERATO HYDARA HEWITSON, WITH SPECIAL REFERENCE TO THE SOCIAL USE OF COLOR

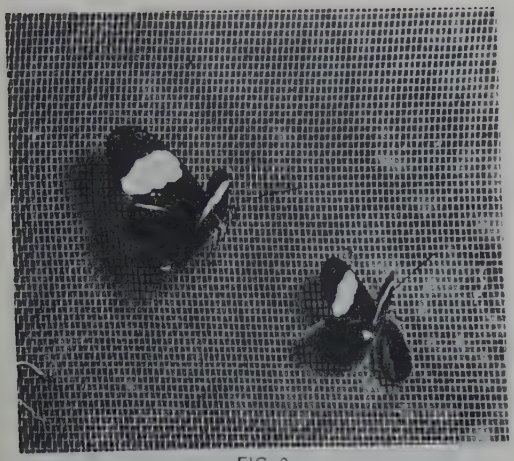

FIG. 9

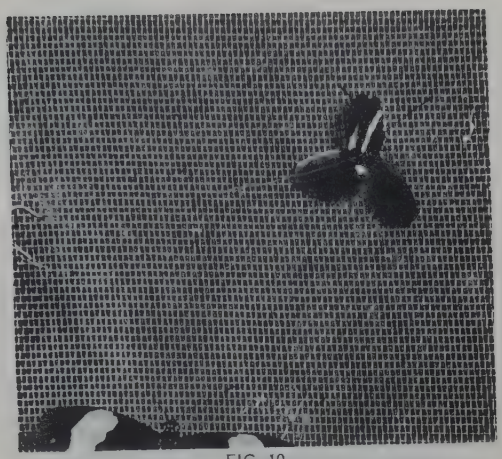

FIG. 10

FIG. 11

FIG. 12

FIG. 13

FIG. 14

IMAGINAL BEHAVIOR OF A TRINIDAD BUTTERFLY, HELICONIUS ERATO HYDARA HEWITSON, WITH SPECIAL REFERENCE TO THE SOCIAL USE OF COLOR

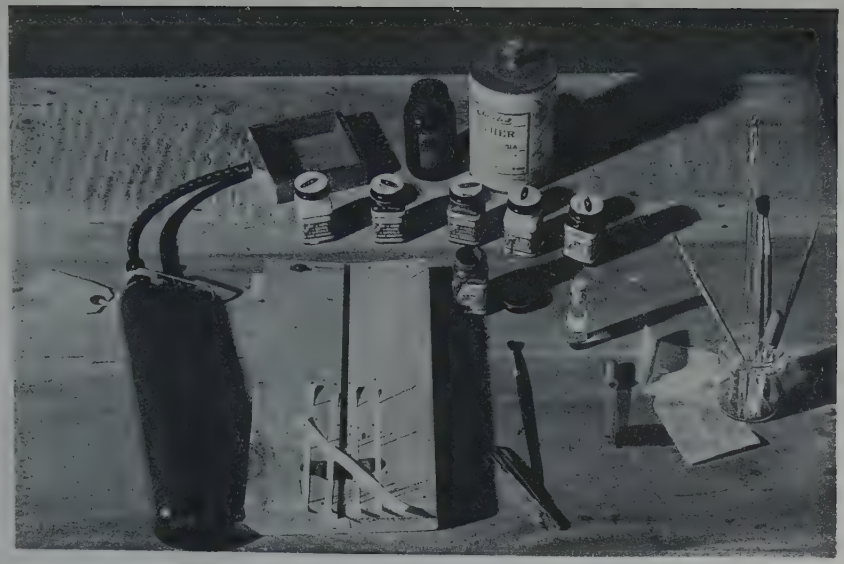

FIG. 15

FIG. 16

FIG. 17

FIG. 18

IMAGINAL BEHAVIOR OF A TRINIDAD BUTTERFLY, HELICONIUS ERATO HYDARA HEWITSON,
WITH SPECIAL REFERENCE TO THE SOCIAL USE OF COLOR

17

Three More Gymnotid Eels Found to Be Electrogenic

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(Plates I-III)

IT has been reported previously (Lissmann, 1951; Coates *et al.*, 1954; Coates, 1954) that three species of knifefishes, Family Gymnotidae, other than the well-known Electric Eel, *Electrophorus electricus,* are electrogenic. Three more species, never before regarded as electrogenic, are here reported as producing electric discharges more or less continually, and apparently in patterns peculiar to each species.

The three species are *Apteronotus albifrons,* of which two individuals were examined, *Steatogenes elegans* and *Sternarchus oxyrhynchus.* All three were found to emit electrical impulses of low amplitude and great regularity.

The discharges were measured by electrodes inserted into the fresh water in which the fishes were swimming freely, connected to a cathode ray oscillograph. The temperature of the water was 25° C. and the water was that to which the fishes were accustomed. The electrodes were insulated to their tips, which were silver, and were spaced at varying intervals as indicated in the figures. They were held as close to the test fish as possible without disturbing it, both when the fishes were swimming and when they were lying quietly at the bottom of the tank. Voltages measured in our experimental conditions did not exceed 400 millivolts.

A. albifrons was represented by two individuals, one of which was 215 mm. long and had been in captivity for more than two years. This specimen was presumed to be adult. The other was 50 mm. long and was obviously immature. Oscillographic traces of the discharges of these are shown in Plate I, Figs. 1 & 2. The regularity of the discharges of the adult is obvious, in contrast to the irregularity and lack of pattern of the other. There was no apparent difference between recordings made while the fish were either resting or swimming.

The pattern of discharge exhibited by *Steatogenes elegans,* Plate I, Figs. 3 & 4, showed that it discharged regularly while resting (Fig. 3) and equally regularly, but about four times as fast when it was deliberately disturbed, (Fig. 4). This is an indication that the frequency of the discharges is centrally controlled.

Plate II shows the varying magnitude of the discharges of *A. albifrons* when the electrodes were placed near different parts of the body. This suggests that the organ producing the discharge is located in the caudal extremity, as might be expected in this family. The extreme regularity of the discharges is quite apparent in these figures.

The discharge of *Sternarchus oxyhynchus,* Plate III, Figs. 11 & 12, shows the same regularity and almost the same frequency as that of *A. albifrons,* that is, about 1,000 discharges per second. Such high, steady rates of discharge are rarely encountered in any physiological systems.

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EXPLANATION OF THE PLATES

PLATE I

Oscillographic traces of discharge of *Apteronotus albifrons.* Time scale (lower line), 1,000 c. p. s.

FIG. 1. Adult fish.

FIG. 2. Immature fish.

Oscillographic traces of discharge of *Steatogenes elegans*. Time scale (lower line), 100 c. p. s.

FIG. 3. While resting.

FIG. 4. When disturbed.

PLATE II

Localization of discharge of *Apteronotus albifrons*. Time scale (lower line), 1,000 c. p. s.

FIG. 5. Drawing showing segments measured on oscillograph.

FIG. 6. With electrodes on "A" in drawing.

FIG. 7. With electrodes on "B" in drawing.

FIG. 8. With electrodes on "C" in drawing.

PLATE III

Oscillographic traces of discharge of three species, with electrodes 2.3 cm. apart.

FIG. 9. *Apteronotus albifrons*, slow sweep.

FIG. 10. *Apteronotus albifrons*, fast sweep.

FIG. 11. *Sternarchus oxyrhynchus*, slow sweep.

FIG. 12. *Sternarchus oxyrhynchus*, fast sweep.

FIG. 13. *Steatogenes elegans*, slow sweep.

FIG. 14. *Steatogenes elegans*, fast sweep.

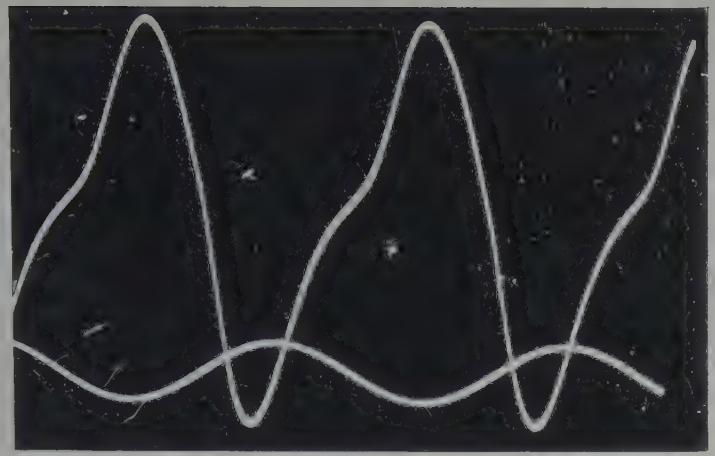

FIG. 1

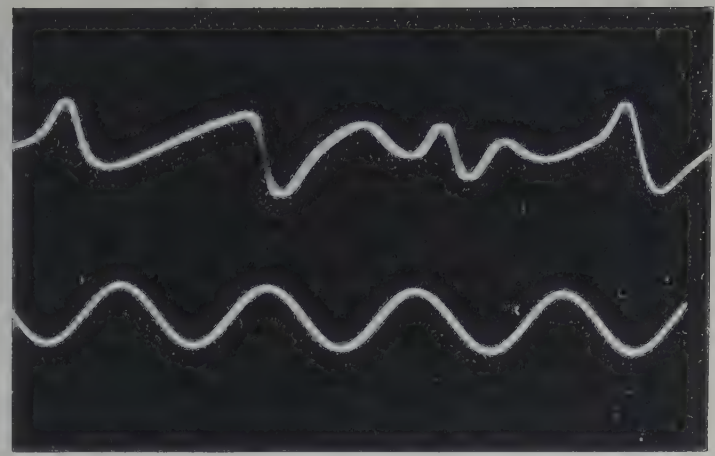

FIG. 2

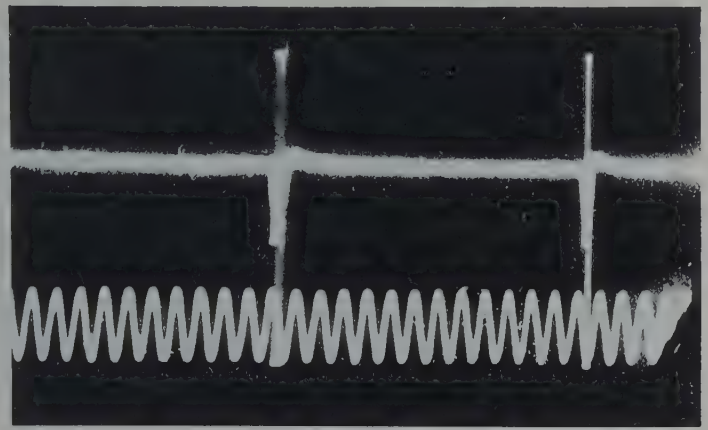

FIG. 3

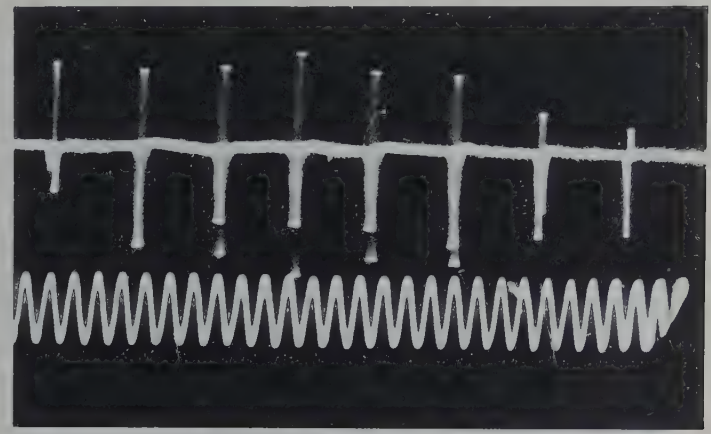

FIG. 4

THREE MORE GYMNOTID EELS FOUND TO BE ELECTROGENIC

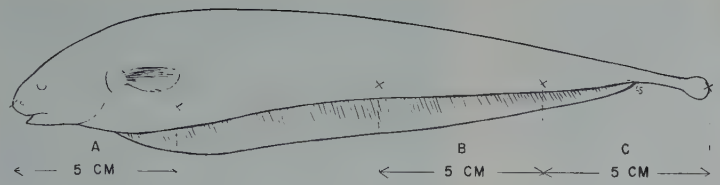

FIG. 5

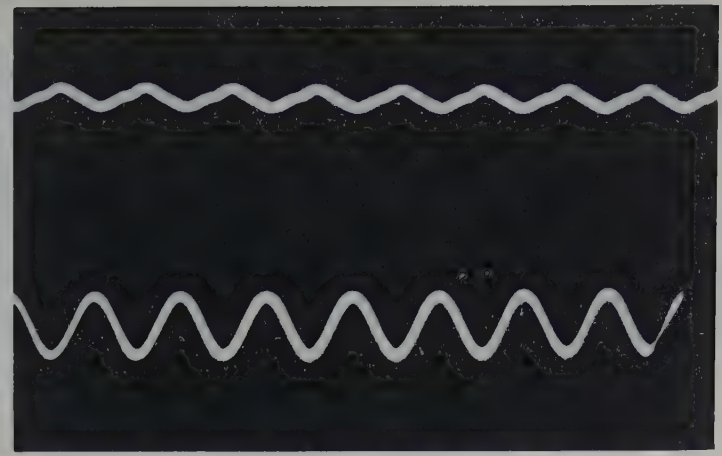

FIG. 6

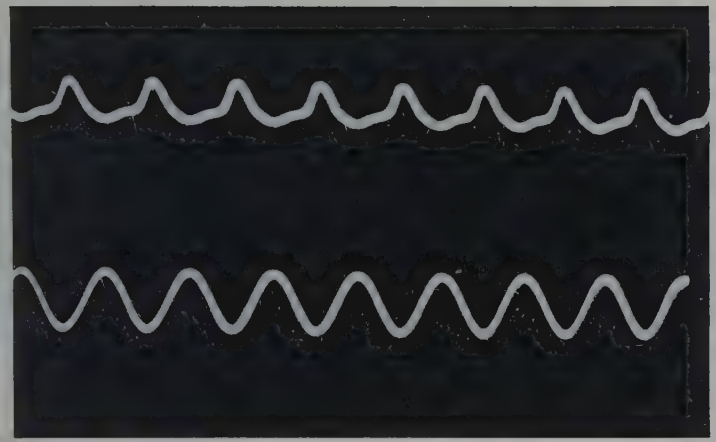

FIG. 7

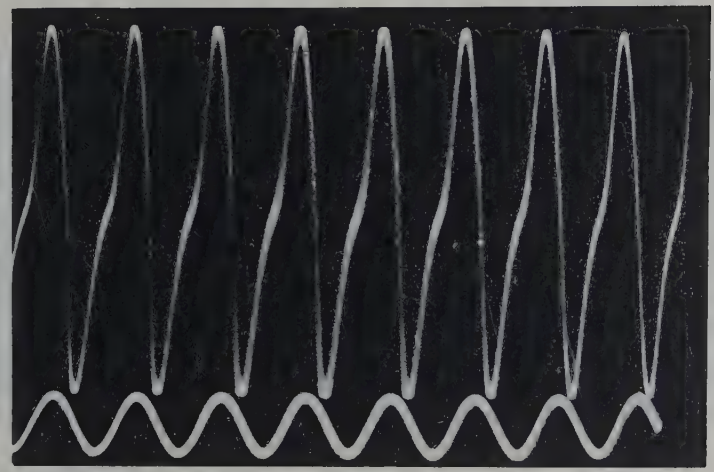

FIG. 8

THREE MORE GYMNOTID EELS FOUND TO BE ELECTROGENIC

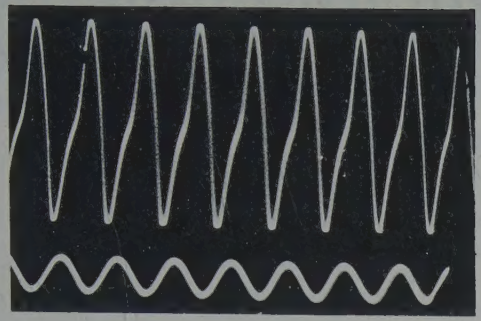

FIG. 9

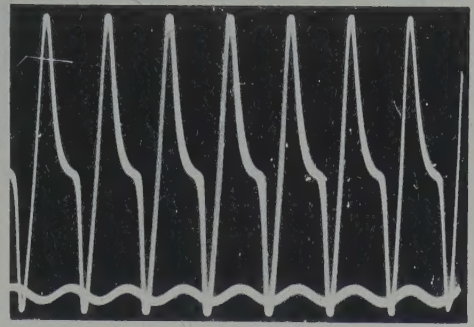

FIG. 11

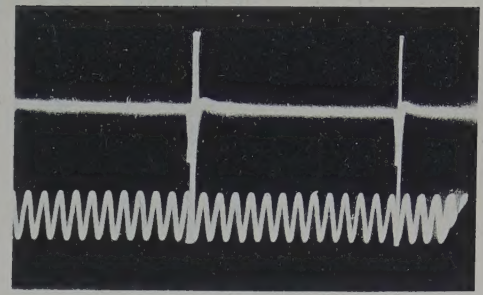

FIG. 13

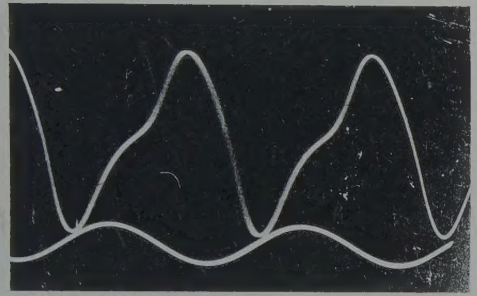

FIG. 10

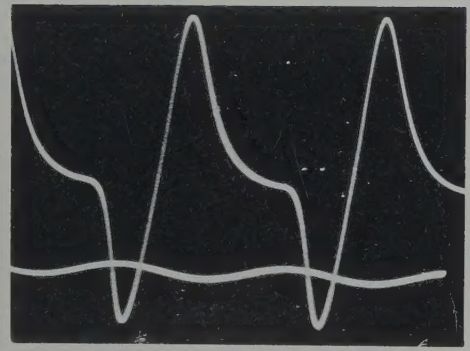

FIG. 12

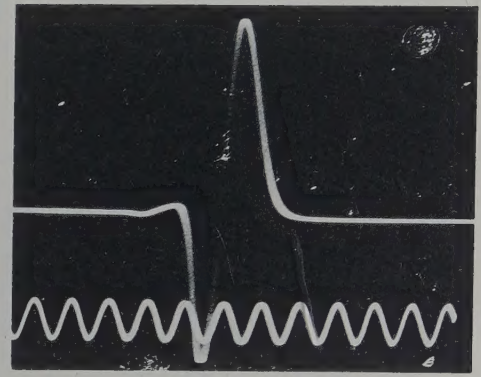

FIG. 14

THREE MORE GYMNOTID EELS FOUND TO BE ELECTROGENIC

INDEX

Names in **bold face** indicate new genera, species or varieties; numbers in **bold face** indicate illustrations; numbers in parentheses are the series numbers of papers containing the plates listed immediately following.